ENVIRONMENTAL POLLUTION BY MISSILE PROPELLANTS

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Contract Monitor: Philip Diamond
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(Prepared under Contract No. AF 33(616)-7801
by
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Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio Rpt. No. MRL-TDR-62-38. ENVIRONMENTAL POLLUTION BY MISSILE PROPELLANTS. Final report, Apr 62, viii + 112p. incl. illus., tables, 44 refs. Unclassified report

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3. Contamination
4. Soils
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FOREWORD

This study was initiated by the Biomedical Laboratory of the 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was administered by the Texas A. and M. Research Foundation, College Station, Texas under Contract No. AF 33(616)-7801. Actual research was performed by the Biology, Soil and Crop Sciences, and Plant Sciences Departments of the A. and M. College of Texas, College Station, Texas. Dr. Walter W. Heck, of the Plant Sciences Department, was the principle investigator. Mr. Philip Diamond, of the Toxic Hazards Section, was the contract monitor for the 6570th Aerospace Medical Research Laboratories. The work was performed in support of Project No. 6302 entitled "Toxic Hazards of Propellants and Materials," and Task No. 630204 under the title of "Environmental Pollution." The research sponsored by this contract was started in February 1961 and was completed in November 1961.

The authors acknowledge the assistance of Mr. Eddy Flinn and Mr. Lloyd Hayes in many facets of the technical work involved in this report.

This report is cataloged by the Texas A. and M. Research Foundation as Project RF-280.

The following areas were investigated by: Dr. Walter W. Heck, Effects on Plant Growth and Development; Dr. Luther S. Bird, Plant Physiology and Pathology (Effect on soil bacteria and fungi); Dr. Morris E. Bloodworth, Soil Physics (Effect on soil, soil structure, and run-off water); Dr. William J. Clark, Limnology and Phycology; and Dale Darling and Martha B. Porter, Experimental Work and Literature Survey.

ABSTRACT

The effects of 21 missile fuel components, in concentrations of 0, 1, 10, 100, and 1000 ppm, were determined on aquatic organisms (goldfish and Daphnia) soil microflora (bacteria, actinomycetes, and fungi), plants (squash, soybean, cotton, cowpea, and corn) and soils (leachability, runoff, and structure).

Some or all of the aquatic organisms died when exposed to 100 ppm of the test chemicals. The same concentration of chemicals had no effect on the counts of soil microflora. Three of the test samples may sterilize the soil of actinomycetes. A concentration of 1000 ppm of two of the compounds and two ionic species produced inhibition of seeding germination. Three of the ionic components, when used as a soil drench at 100 ppm, produced toxic symptoms. When three of the test chemicals (gases) were used at 100 ppm as air pollutants, severe injury or death occurred in all species. Soil analyses were erratic and indicated further research on this problem. Future studies should include the concentration-time relationship of the toxic materials and the absorptive capacities of soil fractions for the test compounds.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

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I

INTRODUCTION

The newer high energy missile propellants are, in varying degrees, toxic substances. Since the effects of these substances on the local environment are largely unknown, it is necessary that some understanding of their potential effect be obtained during the planning stages of large missile operations.

This project was devised to study the effects of some of these toxic fuels and their combustion products on the local environment and to evaluate these materials as air, water, and soil contaminants. The initial phases of this project were of an exploratory and survey nature. Twenty-one test chemicals were included in this survey. Initially, these compounds were submitted to a rather comprehensive literature survey which delineated those compounds which had been well studied from those which had been poorly studied or not studied at all. Following the literature review, a survey research program was set up in four major areas.

The first research area was a study of the effects of the test chemicals on the soil microflora. Each chemical was added at 100 ppm (by weight) to the test soil. After a period of incubation the actinomycete, fungal and bacterial populations were determined for each treatment.

The second area included the interaction of each test chemical with a specific soil type. This study included the leachability of each test chemical, the effect of each chemical on soil structure and the effect of runoff water on the transport of chemical compounds from surface soil area.

The third area encompassed the effects of the test chemicals on plant growth and development. This area was sub divided into a study of seed germination and development; the effects of each chemical when added as a soil drench to the soil in which seedling plants were growing; and, the effects of several gaseous compounds injected into growth chambers on the growth of seedling plants. Five test plants (squash, soybean, cotton, cowpea and corn) were used in these studies.

The last area studied included the effects of the test chemicals on aquatic life. Each chemical was tested at 0, 1, 10, 100 and 1000 ppm on goldfish and Daphnia. These test runs were made over a three-day period.

The results are discussed in general terms and recommendations for future research are outlined.

II

REVIEW OF LITERATURE PERTAINING TO ENVIRONMENTAL POLLUTION

This review presents detailed results of experimental studies, pertinent to environmental pollution, conducted with all test compounds. Several compounds have been extensively studied while others show no work, from a pollution standpoint. Even the compounds which have been well studied have been poorly investigated as to their effects on soil, soil structure and runoff water.

The details presented in this review, see bibliography, have been compiled directly from other review articles. At no time was the original literature reviewed.

The compounds are discussed individually or by groups. All detailed information has been tabulated for easy reference and is presented in Table A-1 of the Appendix.

A. Fluorine:

Fluorine gas is so reactive under atmospheric conditions that it probably reacts to produce hydrogen fluoride (HF) before reaching vegetation, soil or aquatic areas (39).

$$F_2 + H_2O + O_2 \longrightarrow 2HF + O_3$$

Evidence has been reported suggesting that silicon tetrafluoride (SiF_4) produced plant injury of a nature similar to that produced by HF. A third gaseous fluoride, fluosilicic acid (H_2SiF_6), which has been reported in field studies (29), hydrolyzes to HF under atmospheric conditions. Thus Faith's statement (15) that HF is the only toxic fluorine containing gas is probably fairly accurate.

Hydrogen fluoride will produce markings on the leaves of some plants at a concentration of 1 ppb over a period of several days (2). No other pollutant studied is this toxic.

The nature of HF lesions seems to depend on the concentration used. Higher concentrations (about 1 ppm) for several hours produce interveinal and marginal acute markings similar to injury shown by sulfur dioxide (SO_2). Sensitive plants fumigated at 1 to 50 ppb for several hours produce less severe lesions, which are similar to those found in field studies (29). It is difficult to define field symptoms for all plants since the symptoms vary with species. However, the field symptoms are quite characteristic for a given species.

1. Typical injury symptoms: (39,43,44)

a. Killing of marginal areas or tips of leaves.

b. Spotting and killing of interveinous tissue.

c. Initial symptoms are often the development of gray-green areas along the margins of the leaves. These areas become straw-colored after standing 3 or so days.

d. Several species develop a brown or dark brown color several days after fumigation.

e. Some plants show fresh lesions each day for as long as a week following moderate fumigation.

f. Leaves are rarely uniformly marked. Often only one side will be marked.

g. Leaves of some pome fruit trees lose the dead margins leaving ragged edged but healthy appearing leaves

h. Sensitive monocotyledons usually show leaf tip injury. This may be accompanied by streaking or spotting of the leaves.

i. Pine needles may be shortened, proportional to severity of exposure, and may be shed in 1 to 2 years.

j. Generally young mature leaves are most sensitive, followed by mature leaves with the youngest leaves showing the greatest sensitivity.

2. Environmental conditions and HF sensitivity: (18,44)

a. Withholding water from plants for a period previous to fumigation develops resistance to HF, even though they have adequate water at the time of fumigation.

b. The fluoride content of leaves increases during the growing season.

c. There is some disagreement as to whether high humidity or the wetting of plant surfaces increases HF injury and/or absorption.

3. General comments on HF sensitivity: (19,39,40,43)

a. Gladioli show a range of sensitivity between different varieties. Species of gladioli vary in their ability to absorb and tolerate HF.

b. Fumigation of Jerusalem artichoke for 4 hours with 0.5 ppm HF produced plant injury. Sweet potato and tomato were twice as sensitive while peach and buckwheat were four times as sensitive as the artichoke.

c. There is no evidence of "hidden injury" to the tomato by HF fumigation.

d. Gladioli, fruit trees, barley, alfalfa and cotton show a temporary reduction in photosynthesis without visible injury. With visible

injury the photosynthetic rate is proportional to the percent of intact tissue.

e. Insoluble fluoride compounds deposited on the surfaces of leaves would probably cause no damage since they would not be absorbed.

f. Terminal injury and the fluoride content of the leaves is about the same whether the element is added from the air or the soil.

g. Plants grown in nutrient solutions containing fluoride show maximum injury when optimum concentrations of nitrogen, calcium and possibly potassium are also present. Plants growing under these conditions also seem to be more susceptible to HF, but the nutrient has less effect if the fluoride is absorbed from the air rather than from the nutrient solution.

h. Plants show a wide range of tolerance to soil and air-born fluorides. Reasons for this tolerance are not clear but several factors probably are important: fluorides are translocated to leaf margins where their concentration may be as great as 100 times the concentration in the main body of the leaf; insoluble fluoride salts may be formed in the tissues, thus being inactivated; and, fluorides may form organofluorides in the tissues, which could increase or decrease tissue sensitivity.

i. Some plants, especially forage plants, may contain fluoride concentrations in excess of 50 ppm with no visible plant injury. Livestock fluorosis may develop in animals using foliage containing this high fluoride concentration.

4. Interactions of soil and fluoride on plant growth: (9,27,39)

a. Soluble fluorides added to soil tend to form Ca salts, especially in alkaline soils. These salts are relatively insoluble and thus less toxic to plants.

b. Addition of calcium, magnesium or sodium fluoride to soil at 300 lbs/acre produces no plant injury. Equivalent incorporations of hydrogen fluoride and potassium fluoride produces some effects on plants. However, even 800 lbs/acre of hydrogen fluoride has little effect on plant growth while 500 lbs/acre of potassium fluoride is repressive, and even lethal, to several crops on highly acidic soils. The effect of hydrogen fluoride and potassium fluoride are markedly modified when the same soils have been limestoned.

c. No increase of fluoride, in plants, has been noted where rates of application were below 0.5 g of fluoride per 6-7 kg of soil.

d. The degree of toxicity and the amount of fluoride absorbed by buckwheat and tomato plants, which were grown on Sassafras loam and sandy loam at various pH and fluoride levels, were reduced as the pH was increased.

e. Injurious effects from comparable fluoride treatments were of greater severity on the coarse textured sandy loam soil than on the medium textured loam.

f. Air-borne fluorides did not affect the fertility of a rock-divided soil nor the uptake of fluoride from this type of soil.

5. Effects of fluorides on soil and soil structure: (7,27)

a. Topsoil in some industrial areas may contain as much as 1640 ppm fluoride.

b. Stimulated fluoride washdowns from rainwater of up to 2 lbs/acre/year were retained completely by each of 4 unlimed soils against 6-years' rainfall.

c. In 1-year and 12-year lysimeter experiments, 5000 lbs/ acre inputs of calcium fluoride onto a 7-inch layer of limestoned soil were retained almost entirely against leaching from precipitations of 51 inches/year. In contrast, fluoride proved readily leachable from the soil when that element was a component of incorporations of calcium silicate slag.

d. After 4 years leaching from 50 inches annual rainfall, the fluoride retention from 200-800 lbs/acre additions as hydrogen fluoride were as much as 99.5% of the inputs.

e. Retention of additive fluoride by acidic soils in the lysimeters were proportionate to the soil content of aluminum oxide.

f. Stability of calcium fluoride is indicated by the meager release of its fluoride and calcium components to rainwater leachings. In contrast was the mobility of the silico fluoride engendered in slagged soils.

g. The more fluoride fixed, the finer the soil texture.

6. Effects of fluorides on fish: (17)

Fluoride has an effect on the condition of the teeth of fish living in fluoride-contaminated water. This relationship in higher animals and man is well documented.

B. Chlorine: (4,8,15,17,29,31,40,43)

Chlorine is found in polluted atmospheres as free chlorine (Cl_2); as hydrochloric acid (HCl); as chlorine containing organic compounds; and, as inorganic chlorides (salts). Chlorine gas is more toxic to vegetation than sulfur dioxide by a factor of 2 or 3.

Chlorine gas is much more toxic to land plants as an air pollutant than when in water solution. It takes less than 1 ppm by volume of Cl_2 to spot more sensitive plants whereas it requires several ppm by weight

in water to injure these plants.

The most characteristic plant response is the spotting of leaves. Dead areas develop between the veins of broadleaved plants, generally toward the center of the leaf. These first appear as cooked areas but turn straw-colored to brown after a few days. The distinctive feature of chlorine injury is the mottled appearance of the marked leaves. The tissue directly over the veins remains green while chlorosis develops on either side of the veins.

A "silver-leaf" effect of the upper epidermis has been observed in sugar beets.

As with HF, middle-aged leaves are the most susceptible to Cl_2 injury followed in order by the oldest and then the youngest leaves. Wilted plants show pronounced resistance to Cl_2 injury.

Some species, not specifically sensitive to the chloride ion, may accumulate 4% chloride or more without any specific burn symptoms. However, species which are quite salt sensitive may develop leaf-burn symptoms which are not specifically related to chloride accumulation.

Three types of resistance to the oxidizing action of Cl_2 are recognized: ordinary vegetative cells with a waxy envelope, ordinary vegetative cells with a waxy substance, and spores.

Chlorine can be used to destroy algal blooms. The action of Cl_2 upon bacteria differs greatly with the character of the organism. Bacillus tuberculosis is very resistant. The spores of all bacterial forms are unattacked except by excessive doses of Cl_2.

When dissolved in water, chlorine hydrolyzes immediately to:

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^-$$

At concentrations of total Cl_2 below 1000 ppm no measureable quantity of chlorine (gas) exists in solution. The undissociated form, HOCl, appears to be the primary toxic principle and the bactericidal agent in the use of chlorine for disinfection. Some waters become toxic to freshwater fish, on addition of HOCl, without lowering the pH as low as 5.

The wide discrepancy in the tabulation of the effect of Cl_2 on fish is evidence of the fact that pH, temperature, dissolved oxygen, and the synergism and antagonism of other pollutants markedly effect the toxicity of free chlorine (or HOCl) to fish.

C. Bromine:

No reports were found on the effects of bromine on plants, aquatic life, or soils. Possibly bromine will affect plants in a manner similar to chlorine. However, a higher concentration of bromine would possibly be needed to cause equivalent damage.

D. Perchlorates: (34)

No information was available on the effects of NO_2ClO_4 and NH_4ClO_4, although many workers have investigated the toxicity of ammonium compounds.

Stewart and Leonard reported that perchlorates are toxic to citrus and occur as traces in some natural nitrate materials.

E. Acids:

1. Introduction: (3,13,26)

Acid-sensitve plants can be divided into three groups:

a. those that fail to grow on acid soils because of the high concentration of free aluminum ions, such as barley and wheat;

b. those that are adversely affected by the high concentration of hydrogen ions, such as rape and poppy; and,

c. those that are indirectly influenced by the hydrogen ion concentration, which prevents the development of symbiotic bacteria, such as peas.

Acids and alkalis can destroy bacteria and other organisms. They are also lethal to fish and other forms of aquatic life. In particular, fish can be adversely affected if suddenly transferred from an alkaline stream to an acid stream or vice versa.

Hydrogen ion concentrations greater than .00001 N (pH 5) apparently cause a coagulation of gill secretion and asphyxia, or this pH may exert an astringent or corrosive effect upon the gill tissues with a similar result. In general, under otherwise favorable conditions, pH values in the range from 5.0 to 9.0 are not lethal for most fully developed freshwater fish.

Strong mineral acids (i.e. H_2SO_4, HCl and HNO_3), H_3PO_4, and some moderately weak organic acids are lethal to fish only when the pH drops below 5. The differences between the toxicities of these acids, which have been reported, are probably due to the acid anions.

A number of weak inorganic and organic acids (i.e. H_2S, HOCl, HCN, H_2CO_3, chromic, tannic, H_2BO_3, sulfurous, benzoic, acetic and propionic) may be toxic to fish above pH of 5. Toxicity above this pH is due chiefly to the undissociated acid or acid gas in solution. In some cases the anion or salt may be the toxic principle. Several of these acids (especially the first three) are lethal at very low concentrations (see Table A-1).

2. Nitric acid: (15,17,26,29,31)

The symptoms of plant injury due to nitric acid vapor include brown to brownish-black spots on the leaves. The blades of grain

plants and the needle tips of conifers may assume a bright yellow color. Concentrations of 25 ppm will cause these effects.

Nitric acid (vapors or liquid) on contact with water or soil will react immediately with various metal salts to form metal nitrates. Thus, the free acid would not long exist as such after spillage on soil or in aquatic areas.

Algal blooms are associated with the presence of fairly high concentrations of certain nutrients including nitrates, which stimulate the growth of algae. Eventually the algae undergo decomposition and so can cause damage to fisheries by oxygen depletion. On the other hand, high nitrate concentration in water can stimulate the growth of plankton and aquatic weeds. By increasing plankton growth and thus the development of fish food organisms, nitrates indirectly foster increased fish production.

Excessive nitrate tends to reduce soil permeability. Nitrates may accumulate to toxic concentrations in the soil solution but their effect is usually osmotic.

Nitric acid dissociates in water into hydrogen and nitrate ions with a resulting tendency to lower the pH value. Its principle deleterious effect on fish, therefore, is its acid reaction. In water very slightly acid with nitric acid fish will not only neutralize the acid but will make the water alkaline.

3. Nitrous acid: (17)

Little work has been done on the effect of nitrous acid on plants, aquatic life or soils. Nitrous acid will form metal nitrites on contact with water or soil. The nitrite ion is quickly oxidized to nitrate and thus is seldom present in soils or aquatic areas. Under anaerobic conditions, as in sewage contaminated areas, nitrite may accumulate to several ppm.

Nitrites have been found to stimulate the growth of plankton in reservoirs.

4. Hydrochloric acid: (17,29,39,40)

Hydrochloric acid (HCl) is considerably less toxic to vegetation than sulfur dioxide. The threshold concentration is about 10 ppm for a few hours fumigation. The older literature indicates that about 50 to 100 ppm is required to cause injury but determinations were done in a static atmosphere.

The chloride ion tends to travel to the margins of the leaves where it accumulates causing a chlorotic margin which may become necrotic. At higher concentrations, lesions similar to acute sulfur dioxide marking are produced. The lesions are found principally on the margins or tips of the leaves but sometimes between the veins as well.

This strong acid is highly soluble in H_2O, dissociating to hydrogen and chloride ions. The principle effect of hydrochloric acid lies in changing the hydrogen ion concentration. It is the resulting pH rather than concentration of hydrochloric acid that governs lethality toward aquatic life; hence, the discrepancies in the reports of lethal doses of hydrochloric acid to fish.

5. Nitrogen dioxide: (2,25,32,40)

Nitrogen dioxide reacts with water vapor, water, or soil water to form nitric and nitrous acids:

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$$

These will produce the metal salts. Thus, it is not possible to study the direct effects of this acid on aquatic or soil organisms. The direct effect of this gas on plants is also probably a nitric and nitrous acid effect.

Two main types of markings are produced on plants by the oxides of nitrogen (including nitrogen dioxide) - the usual collapsed dead tissue and a waxy shiny green coating on the leaves. The collapsed tissue markings consist of irregular shaped areas generally located between the important side veins but usually closer to the margin than the midrib. Middle-aged, fully expanded but not yet senescent leaves are the most sensitive. The youngest leaves always show the least amount of marking. The color of the markings is usually white to tan or dark brown. The waxy polished green markings, not associated with killed tissue, have been observed on 50% of the species. The occurrence of this symptom is not associated with the age of the leaf. It occurs on both surfaces of the leaves of some plants and the upper surface only on other plant leaves. The symptom usually disappears about a week after exposure.

Considerable work has been done the past few years on the indirect effects of the oxides of nitrogen (including NO_2) on plant growth. These studies have developed from a study of the Los Angeles-type smog or photochemical smog. The oxides of nitrogen are strong oxidizing agents and react with certain unsaturated hydrocarbon gases or vapors to produce an extremely toxic organic oxidant. This effect is much stronger in the presence of light and the following reaction pathway has been proposed, where hv refers to light energy:

$$NO_2 + hv \longrightarrow NO + O$$

$$O + O_2 \longrightarrow O_3$$

$$NO + O_2 \longrightarrow NO_3$$

$$NO_3 + O_2 \longrightarrow NO_2 + O_3$$

The quantum efficiency below 370 mu for the dissociation of NO_2 is close to unity. Thus, there is a self-perpetuating system produced.

The ozone formed reacts with olefinic hydrocarbons to produce the injurious organic oxidant(s). Nitrogen dioxide per se is not nearly as toxic as in combination with certain hydrocarbon gases.

6. Hydrocyanic acid: (3,8,19,26,29,37)

Hydrocyanic acid or hydrogen cyanide inhibits both growth and respiration of plants. The respiration of young plant tissues (carrot leaves, barley seedlings) becomes less sensitive to hydrogen cyanide as they mature.

Experiments with orange trees indicate that absorption and injury are greater in the day than at night, and well-irrigated plants are more sensitive than drier plants.

The highly toxic constituent of illuminating gas is hydrogen cyanide. The injury due to passing illuminating gas through soil pots decreased in the following order: tomato, willow, maple, cherry, silver bell and privet. With a slow gas flow, it is probable that hydrogen cyanide would be disposed of by microorganisms in the soil. A comparison shows that the tomato plant is injured to about the same degree whether it is in the soil while illuminating gas flows through the soil or is set in the soil immediately after gassing.

Hydrogen cyanide is formed by sodium cyanide, potassium cyanide, and other soluble cyanides in water. The extent of this hydrolysis is greatly influenced by the pH of the solution.

Under natural conditions cyanides deteriorate or are decomposed by bacterial action, so that excessive concentrations may be expected to diminish with time.

In solutions of hydrogen cyanide and soluble cyanides, the toxicity may be ascribed to the action of undissociated molecules of cyanide but probably is due to the cyanide anion. Cyanide enters the body of a fish through the gills and lining of the mouth, circulates in the blood stream and interferes with enzymatic oxidative processes in the body cells.

At low concentrations (less than 1 ppm of cyanide) fish may survive a long time in a condition resembling anaesthesia; they lie almost motionless on their sides breathing very slowly and feebly. When fish are poisoned by cyanide, the gills become considerably brighter in color than those of normal fish.

Wuhrmann and Woker (from Klein, 26) found that the toxicity of cyanide to Chub was almost doubled on changing the pH from 8.8 to 7. Southgate found that potassium cyanide solutions have approximately the same toxicity to trout over the pH range 6.0 to 8.5.

The average correlation coefficient between the periods of survival of individual rainbow trout between 0.125 and 0.175 ppm of

cyanide was 0.749. In other experiments susceptibility to cyanide poisoning (at 0.15 ppm) increased significantly with increase in length of the fish.

The period of survival of rainbow trout decreased with rising temperatures in solutions containing from 0.3 to 1.0 ppm of cyanide. With slightly lower concentrations of cyanide (0.175 to 0.25 ppm) temperature had no apparent effect. In H_2O containing 0.125 and 0.15 ppm of cyanide, increasing the temperature appeared to prolong the period of survival.

The toxicity of cyanide is greatly increased by a fall in concentration of dissolved oxygen, even by small reductions at O_2 concentrations near the saturation value.

F. Amines and Bases:

Strong alkalies probably produce asphyxiation in fish by coagulation of gill secretions, similar to the action of strong acids.

1. Unsymmetrical dimethyl hydrazine:

There is no information available about the effect of unsymmetrical dimethyl hydrazine on plants, aquatic life or soils.

2. Hydrazine: (6,37)

There is very little information available about the effects of hydrazine on plants, aquatic life, or soils. Hydrazine strongly inhibits the growth of Cucurbita pepo. Fingerling rainbow trout immersed in concentrations of hydrazine hydrate as low as 0.7 ppm lost equilibrium in less than 24 hours.

3. Ammonia: (2,5,13,37,40)

Ammonia gas is toxic to most plants. Ammonia tends to cause cell collapse and death of plant tissues, more toward the margin and tips of the leaves than toward the center. There is no relationship between leaf veination and the location of the markings. Several hours after exposure to ammonia gas the leaves become discolored, changing from a green to a cooked green appearance, becoming brown on drying. Sometimes the color varies from a dark-brown tan to almost a silvery appearance. Very often the spots are small and very close together so that the overall appearance of marked leaves is a powdery one. Fully expanded leaves, but not the old leaves, are most sensitive. Partially opened leaves are very resistant and are only rarely marked.

Ammonia at concentrations up to 22 percent did not show a sterilizing effect on the soil with regard to the bacterial microflora. The number of fungi, however, were decreased in the areas of application.

Solutions of ammonia or ammonium hydroxide, and also ammonium salts, prepared with natural water, can be very toxic to fish even

when the pH is below 9. The toxicity of these solutions evidently is dependent largely, if not entirely, on the concentration of ammonia (NH_3). The periods of survival of trout in a given concentration of NH_3 increased as the concentration of dissolved oxygen was increased, and at each concentration of dissolved oxygen the period of survival decreased as the concentration of NH_3 increased. The effect of oxygen, in increasing survival times, was greater at the lower concentrations of NH_3.

Field experiments have shown that the relationship between the concentration of NH_3 and the period of survival was approximately the same in the field and in the laboratory.

G. Metal Oxides and Salts: (13,38)

All metal cations can be toxic to fish in rather dilute (less than 0.05M) physiologically unbalanced solutions of single metal salts. The harmful action of some metal salts is believed to be internal or intracellular. The lethality of some metal salt solutions to fish is markedly influenced by temperature, the dissolved oxygen concentration, the volume of experimental solutions in which the fish are held, the purity of the chemicals used, and the length of the tests.

Chloride salts are more injurious to plants than sulfates. Peach trees grew in a culture composed mainly of sulfate salts with an osmotic pressure of 3.6 atmospheres, but died when the culture was composed mainly of chloride salts at 3.4 atmospheres. In another instance, the yield of cotton and 5 other crop plants was reduced by 37 percent by 100 meq./1 of chloride salts and by 43 percent by 200 meq./1 of sulfate salts. Nitrates retard growth at concentrations greater than 64 meq./1 but also increase fruitfulness.

1. Aluminum: (13,22,26,33,41)

The solubility of aluminum varies with the pH and is minimal near neutrality. A pH below 5 insures the solubility of aluminum which is toxic to many plants.

Aluminum in acid soils causes pink hydrangea flowers to turn blue.

Aluminum salts affect fish the same as sodium hydroxide and other strong alkalis, initiating asphyxiation by coagulation of gill secretions. Aluminum is classified as a highly toxic element to fish, although some of its salts may be comparatively harmless in highly mineralized water due to precipitation of insoluble compounds.

The hydrogen ion is more toxic to Polycelis nigra than aluminum.

2. Beryllium: (10,11,21)

Very little information was available on the effects of beryllium on plants, aquatic life or soils.

Beryllium salts in equivalent molar concentrations produce effects similar to lead salts at concentrations of 0.01 percent, above which germination and growth of seed are delayed. Beryllium will inhibit germination of cress and mustard seed for at least 18 days without destroying the vitality of the seed. Beryllium is highly toxic to tomato and Chlorella growth at acid pH's.

Concentrations of beryllium from 9 to 18 ppm interfere with tadpole development although they may survive and grow in a 0.18 to 0.45 ppm concentration for one month.

3. Boron: (17,36)

Symptoms of advanced boron toxicity in trees are leaf yellowing and burning, premature leaf drop, and reduced yield. Symptoms of boron injury may not appear for several years depending on the concentration of the soil solution.

The crops most sensitive to boron are citrus, nut and deciduous fruits; the crops semi-tolerant to boron are truck crops, cereals and cotton; the crops most tolerant to boron are lettuce, alfalfa, beets, asparagus and date palms.

The average leaching losses of boron, in soil columns 40 cm high to which 40 kg/ha borax had been applied, by a total precipitation of 19.7 inches in 5 months were 23.3 percent in neutral sandy soil, 18.6 percent in acid sandy soil, 0.0 percent in neutral loamy soil, 11.6 percent in acid loamy soil, 40 percent in neutral moor, and 29.4 percent in acid moor soil. During a one-year field experiment boron was leached 14.3 percent on a sandy clay loam, 23.6 percent on a sandy loam, 24.9 percent on a loamy sand and 0.0 percent on light clays and clayey loams. In none of the 7 soils was boron found deeper than 1 meter. The fixation of boron increased with increasing pH and content of clay.

4. Lithium:

Very little information was available on the effects of lithium on plants, aquatic life or soils.

H. Summary:

A systematic study of the literature indicates that several of the compounds to be studied have concerned investigators for several to many years. The amount of research which has been done is directly proportional to the amount of damage done as a result of environmental pollution. Where compounds have been poorly studied, they are either newly developed compounds or have been produced in such small amounts that they have not presented a public nuisance or danger.

1. Plant studies:

The following compounds (HF, F salts, B salts) have been intensively

studied. We will plan to spend a minimum of time with these compounds during the initial phase of our experimental work. A second group (Cl_2, HCN and salts, NH_3, HCl) has been fairly well studied but would warrant further study on special aspects of plant injury. A third group (perchlorates, $NaNO_2$, HNO_3, NO_2, Al salts) has been poorly studied and needs further work in many areas. The fourth and last group (Br_2, NO_2ClO_4, NH_4ClO_4, UDMH, N_2H_4, Be salts, Li salts) has not been studied or shows only 1 or 2 preliminary reports.

2. Aquatic studies: using the above four categories:

   a. Cl_2, HCN and salts, Al salts

   b. Br_2, HNO_3, HCl, NH_3

   c. HF, F salts, NO_2^- salts, B salts

   d. perchlorates, NO_2, UDMH, N_2H_4, BF_3, Be salts, Li salts

3. Soil microorganisms: none of the chemicals have been extensively studied in their relation to soil organisms.

4. Soil and soil structure:

   a. HF, F salts

   b. Al salts, B salts

   c. HNO_3 and salts, HCl, HCN and salts, NH_3

   d. Cl_2, perchlorates, NO_2, UDMH, N_2H_4, BF_3, Be salts, Li salts

Many studies, that have been made with the various compounds show conflicting results. This is probably due to some lack in environmental control or the failure of the various investigators to stipulate the exact conditions under which the experimental work was done. We feel the literature review points up the necessity of strict and accurate environmental control of all conditions in order to gather information that can be used to predict the toxic effects of individual toxicants. More study is needed on the interactions of various toxicants. Missile fuels and exhausts will contain several to many of the listed compounds and more severe injury may occur from the combined effects than from any single toxicant.

III

RESEARCH

A. General Areas of Study

This project has been subdivided into four major categories:

1. Soil Microflora: A survey of the effects of the listed chemicals on the bacteria, actinomycete and fungi populations of Houston Black Clay. Results were subjected to statistical analysis.

2. Soil Studies: The soil studies were divided into three areas of investigation:

   a. A study of the leachability of the test chemicals from a soil column.

   b. A study of the effects of the test chemicals on soil structure.

   c. A study of the amount of test chemicals carried in runoff waters.

3. Plant Studies: These were divided into two main areas of investigation:

   a. The effects of the test chemicals on germination and growth of seeds.

   b. The effects of the test chemicals on seedling growth when chemicals were applied as soil nutrients and as air pollutants (three gases).

4. Aquatic Studies: A survey of the effects of the test chemicals on four groups of aquatic organisms.

B. General Experimental Procedures

1. Organisms to be Studied:

   a. Soil microflora: bacteria, actinomycetes, fungi

      Identification was taken to major groups only. No subdivisions were undertaken.

   b. Plants:

      1) Cucurbita maxima, Duchesne - early white bush squash

      2) Glycine soja, Merrx - soybean

      3) Gossypium hirsutum, L. - cotton (Rex)

4) Vigna sinensis, Endl - extra early blackeye cowpea

5) Zea mays, L. - corn

c. Aquatic organisms:

The two principal test organisms were the goldfish, Carassius auratus (Linnaeus), and Daphnia pulex (de Geer). Limited data were also obtained for the Bluegill, Lepomis macrochirus (Rafintesque), and a Dragonfly nymph (family Libellulidae).

The goldfish were obtained from a local bait breeding farm; the Daphnia culture originally came from Carolina Biological Supply Company; the Bluegill were supplied by the Texas Game and Fish Commission; the Odonata were collected from a pond on the A & M College property.

2. Soil Types:

a. Soil and soil structure:

Lufkin fine sandy loam was used in all phases of this study. The location from which the soil was taken was selected by Dr. C. L. Godfrey, Soil Survey and Classification Unit, Soil and Crop Sciences Department. Chemical, physical and mineralogical characteristics of the Lufkin loam are shown as follows:

Texture:	loam
pH:	6.3
Cation exchange capacity:	11.5 meq/100 gms
Exchangeable cations:	meq/100 gms
Ca:	6.0
Mg:	1.6
K:	0.1
Na:	0.4
Organic matter:	1.8%
Bulk density:	1.58 gms/cc
Sand:	47.5%
Silt:	39.4%
Clay (< 2 μ):	13.1%

Clay mineral types:	Montmorillonite, kaolinite and quartz	
Water retention characteristics:	Saturation	43.1%
	1/10 Atmosphere	26.7%
	1/3 Atmosphere	16.5%
	1/2 Atmosphere	15.4%
	3/4 Atmosphere	13.4%
	1 Atmosphere	10.0%
	5 Atmospheres	6.8%
	10 Atmospheres	5.7%
	15 Atmospheres	5.5%

Analyses were made by M. E. Bloodworth and G. W. Kunze as part of Hatch Project 928 of the Texas Agricultural Experiment Station, College Station, Texas.

b. Soil microflora: Houston black clay:

The general characteristics of the A1-1 horizon of the test soil are: 6 percent sand, 36 percent silt, 58 percent clay, 1.5 to 2.5 percent organic matter, 25 to 27 percent calcium carbonate and a pH of 7.5 to 8.0. The soil lots used were obtained from Substation Number 5, Texas Agricultural Experiment Station, Temple, Texas.

c. Plant studies: A peat-perlite potting mix.

This potting mix is a standard mix developed by the Department of Floriculture of this campus (10). It is made by mixing 30 gallons of moist peat (a dry baled Canadian horticultural peat), 30 gallons of moist perlite (a horticultural perlite), one quart of dolomite and 1/2 quart of 12:12:12 standard agricultural fertilizer. This mix was stored and used as needed for seedling studies. For preliminary work with plants, this is an ideal mix because it allows for rapid growth of plants.

3. Water and Nutrient Solutions:

a. Distilled water was used throughout the soil microflora studies.

b. Tap water was used in the soil leaching and runoff studies. Distilled water was used in the soil structure studies.

c. A nutrient solution was used to water the peat-perlite mix in plant growth studies. The nutrient solution used was a modified one-quarter strength Hoagland's solution (20). To this was added 1 1/4 meq. of NH_4NO_3 and a full strength iron-chelate. No trace elements were added. Tap water was used in making up these solutions, pH was not adjusted.

d. Seed germination studies utilized distilled water with no nutrient additions.

e. Water for aquatic organisms was obtained from a fresh water pond and/or by aeration of tap water for 7-14 days to remove the chlorine. The chlorine content of the tap water was periodically checked.

4. Chemicals and Their Use:

a. The following chemicals were studied:

1) Halogens: hydrogen fluoride - HF (not used in seed germination studies), chlorine - Cl_2, bromine - Br_2.

2) Perchlorates: ammonium perchlorate - NH_4ClO_4 (not used in the soil studies).

3) Acids or their salts: sodium nitrite (concentration was calculated on basis of HNO_2) - $NaNO_2$, potassium cyanide (concentration was calculated on basis of HCN) - KCN (not used in aquatic studies), nitric acid - HNO_3, hydrochloric acid - HCl, nitrogen dioxide - NO_2.

4) Amines: ammonia - NH_3 (added as NH_4OH to all water solutions - but concentration calculated on basis of NH_3), hydrazine - N_2H_4, unsymmetrical dimethyl hydrazine (UDMH) - $(CH_3)_2NNH_2$.

5) Metal oxides and salts: aluminum oxide - Al_2O_3, aluminum chloride - $AlCl_3$ (as $AlCl_3 \cdot 6H_2O$, used only in microflora studies), aluminum fluoride - AlF_3, beryllium fluoride - BeF_2 (used only in plant seedling studies), boron trifluoride - BF_3, boron oxide - B_2O_3, potassium borate - $K_2B_4O_7$, lithium oxide - Li_2O, lithium fluoride - LiF, lithium chloride - LiCl (used only in the microflora studies).

b. Concentrations of chemicals used: (Concentrations were based on the molecular weights of the compounds used unless noted in above section.)

1) Soil microflora studies: all chemicals were used at a concentration of 100 ppm in the top 2 inches of soil - based on the oven dried weight of soil in the top 2 inches and the molecular weight of the pure compound (water of hydration was not included). An average dry weight of soil (720 gms) was used

in all calculations. Thus, 0.072 gms of the test chemical were used per soil treatment.

2) Soil leaching studies: all chemicals were used at a concentration of 1000 ppm in the top inch of soil (calculated as above). Average dry weight of soil in this 1-inch layer was 763 gms. Thus, 0.763 gms of test chemical were used per soil treatment.

3) Soil runoff studies: chemicals were used at 1000 ppm, calculated as above. The chemical was incorporated at the head of the runoff bed in an average of 823 gms of dry soil spread over a one square foot area. Thus, 0.823 gms of test chemical were used per soil treatment.

4) Soil structure studies: chemicals were used at 1000 ppm, calculated as above. The chemical was incorporated into the entire soil sample (850 gms). Thus, 0.850 gms of test chemical were used per soil treatment.

5) Seed germination studies: chemicals were used at a concentration of 1000 ppm in water solution (weight basis). Chemicals were added to 25 cc of water for each test petri dish. Thus, 0.025 gms of test chemical were used per petri dish.

6) Seedling studies (soil drench): chemicals were used at a concentration of 100 ppm in water solution (weight basis). Each chemical was added to 18 liters of nutrient solution. Plants were continuously subirrigated by this solution for seven days before being removed from the experimental solution. Thus, 1.8 gms of test chemical were used per treatment.

7) Seedling studies (air pollution): three chemicals (Cl_2, NH_3 and NO_2) were used at 100 ppm (by air volume). Methods for measuring gas flow are outlined under the section on seedling studies. These fumigations were run for three hours.

8) Aquatic studies: four concentrations of each chemical were made; usually 1 liter each of 6000, 600, 60 and 6 ppm (on a weight basis) were made and when added to 5 liters of water in the experimental containers gave concentrations of 1000, 100, 10 and 1 ppm, respectively. On a few occasions the solutions were made up in 500 ml and added to 5.5 liters in the experimental containers, but always giving experimental concentrations of 1000, 100, 10 and 1 ppm. All solutions were made up with the same water as was used in the experiment.

c. Mixing and application of test chemicals:

1) Soil microflora and soil studies: water soluble chemicals were taken up in water and mixed with the given weight of soil or

added as a soil drench. Insoluble compounds were added as a powder to the given weight of soil or added on the soil surface and then wetted with a given volume of water.

2) Plant and aquatic studies: chemicals were used in water solution or as slurries in the case of insoluble compounds.

d. Analytical techniques used for the determination of test chemicals.

The techniques used were obtained from the references listed for each chemical element or compound: aluminum (24), ammonia (16), boron (16), bromine (23), cyanide (14), fluoride (30), lithium (16), nitrate (14) and nitrite (42). The procedures followed for hydrazine and unsymmetrical dimethyl hydrazine were obtained from Mr. Philip Diamond of the Toxic Hazards Section, Biochemical Laboratory, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

5. Growth Chambers:

Four special plant growth chambers have been constructed for plant fumigation studies, using a dynamic air flow system.

The chambers are 48 inches wide x 30 inches deep x 42 inches high with a total volume of 35 cubic feet. They are constructed of a 2 x 2 inch wood frame covered with a 1/8 inch clear plexiglas on the top and sides. A 1 x 6 inch wood frame goes around the lower sides and is attached to a 1/2 inch plywood bottom.

Each chamber is equipped with two 2-inch air inlets in the front corners and two 2-inch air outlets in the back corners. The inlets project up 2 inches into the chambers while the outlets project up 36 inches into the chambers. The outlets have machined orifice plates which fit snugly over the outlet tube for measurement of air flow. Several interchangeable orifice plates are available.

Air flow through the four chambers is maintained by a high pressure blower on the downstream side of the chambers. Air from the laboratory is used in these fumigation studies. The air passes through an 18 x 18 inch, 2-inch deep, carbon filter into a 5-inch diameter duct, over a set of heating coils (installed in the 5-inch duct and used to maintain a high day temperature), over a thermostat (for regulating the heating elements), into the 2-inch inlet ducts, through the chambers, out the 2-inch outlet ducts into a 5 inch diameter discharge duct, through the blower and the air is exhausted through the roof.

Night temperatures were maintained at 71.5 $\pm$ 3.5°F with room air-conditioners. Day temperatures were maintained at 84 $\pm$ 4°F with the thermostat controlled heaters in the 5-inch inlet duct. Although no attempt was made to control humidities, the chambers maintained a

65 ± 13% night humidity and a 57.5 ± 12.5% day humidity as recorded using a wet-bulb thermometer.

Each chamber is equipped with a 3-inch deep galvanized metal pan with a center inlet-outlet for use in subirrigation of the plants with water or a nutrient solution.

C. Soil Microflora

1. Methods and Materials:

Metal pans 13 x 3 x 6 inches were used as containers for incubating the treated soil. Each pan was set up with a 3 3/8 inch layer of untreated soil. This was then covered with a 2-inch layer of treated soil. With the gases and volatile liquids, the soil in the pans was brought to a depth of 5 3/8 inches before watering with drench solutions.

Water soluble chemicals were taken up in 15 ml of water and this was atomized into the soil as it was being tumbled in a small (5-gallon size) concrete mixer. The non-water soluble chemicals were added as powders as the soil was being tumbled in the mixer. The gases and volatile liquids were taken up in a 150 ml of water and this solution was used to drench the upper 2 inches of soil. In each case pre-determined amounts of the chemicals were added to establish concentrations of 100 ppm, by weight, in the upper 2 inches of soil. All pans received 150 ml of water the first day of the test. Each soil treatment was replicated six times.

After the treatments were established, the soils was allowed to incubate two weeks. During this time 150 ml of water was added to each pan on the fifth and the tenth day. Soil samples were taken on the fourteenth day.

Thirty gram soil samples were taken from the 1/2 to 2 inch depth zone of each pan. Each sample was well mixed and a 10-gram sample was used for determining percent moisture. A 12-gram sample was used for the dilution series.

The dilution series was as follows: the 12-gram sample (approximately 10 grams oven dry weight) was placed in a 90 ml blank, this was vigorously shaken for 15 minutes on a mechanical shaker, 10 ml was then transferred to another 90 ml blank, after manually shaking, 1 ml was transferred to a 99 ml blank, and again, after manually shaking, 1 ml was transferred to a 49 ml blank. One ml aliquots of the 1/10,000 dilution were plated for determining fungi populations and 1 ml aliquots of the 1/500,000 dilution were used for determining the bacteria and actinomycete populations. Three plates of each dilution from each soil treatment and soil replication were made. Sterile water blanks, pipettes and aseptic techniques were used for and during the dilution series.

The following medium was used for growing the fungi:

Agar	25 gms
KH_2PO_4	1 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
Peptone	5 gms
Dextrose	10 gms
Rose Bengal, 1:300 dilution	10 ml
Aureomycin	30 ppm
Distilled Water	to 1 liter

The following medium was used for growing bacteria and actinomycetes:

$MgSO_4$	0.3 gm
$CaCO_3$	0.2 gm
Agar	10.0 gms
Potato dextrose agar, commercial	39.9 gms
Peptone	2.5 gms
Carrot juice, commerical	15.0 ml
Yeast extract	0.5 gm
Acti-dione	17.0 ppm
Distilled Water	to 1 liter

The melted medium was cooled to just above the agar solidification point before being poured into the petri plates containing the 1 ml dilution aliquots. After pouring, the plates were gently rotated to thoroughly mix the medium and the 1 ml aliquot. After standing overnight the plates were inverted and incubated at 74-78°F for 10 days. A Quebec colony counter was then used for counting the colonies per plate. The counts were converted to numbers of organisms per gram of oven dry soil.

Three experiments were conducted. Five chemicals were tested in the first, nine in the second and seven in the third experiment. These experiments were analyzed statistically using the randomized block design by the analysis of variants method.

2. Results:

The results obtained in experiment number 1 are given in Table 1. $NaNO_2$ caused a significant increase in bacteria over the control and the other chemical treatments. There were no significant increases in the actinomycete populations. There was an indication that $NaNO_2$ and $AlCl_3 \cdot 6H_2O$ produced increases in the actinomycete populations. No significant or suggested changes in the fungi populations occurred.

TABLE 1. Effects of five test chemicals on the population counts of soil microflora - experiment 1.

Chemicals		Bacteria[1]	Actino-mycetes[2]	Fungi[2]
$NaNO_2$		30.1	20.1	9.6
Hydrazine		19.1	16.9	11.9
Control*		17.4*	8.3*	10.0*
NH_4ClO_4		17.2	8.6	13.9
$LiCl_3$		14.6	11.3	8.2
$AlCl_3 \cdot 6H_2O$		14.2	25.7	11.4
L.S.D.[3]	.01	n.s.d.[4]	-	-
	.05	10.1	n.s.d.	n.s.d.

[1] Ten thousands per gram of oven dry soil
[2] Thousands per gram of oven dry soil
[3] (L.S.D.) Least significant difference
[4] (n.s.d.) No significant difference

The results of experiment number 2 are given in Table 2. No significant changes in bacterial populations occurred although there is a definite suggestion that HNO_3 and KCN may cause large increases. For the actinomycetes $K_2B_4O_7$ caused significant increases over most treatments but not over the control or AlF_3. There was a definite suggestion that KCN, B_2O_3 and Al_2O_3 sterilize the soil of actinomycetes. Again, there were no real or suggested changes in fungi populations.

TABLE 2. Effects of nine test chemicals on the population counts of soil microflora - experiment 2.

Chemicals	Bacteria[1]	Actino-mycetes[2]	Fungi[2]
$K_2B_4O_7$	20.7	26.1	8.7
Control*	19.5*	13.2*	3.4*
AlF_3	14.8	13.0	4.0
Li_2O	15.1	9.8	3.9

(continued)

(continued)

Chemicals		Bacteria[1/]	Actino-mycetes[2/]	Fungi[2/]
HCl		30.2	6.4	3.9
LiF		18.7	3.3	3.9
HNO_3		669.1	3.2	3.9
KCN		526.9	0.0	5.0
B_2O_3		8.9	0.0	2.4
Al_2O_3		9.6	0.0	3.5
L.S.D.	.01	-	n.s.d.	-
	.05	n.s.d.	15.1	n.s.d.

1/ Ten thousands per gram of oven dry soil
2/ Thousands per gram of oven dry soil

The results of experiment number 3 are given in Table 3. No real differences in the populations of the three groups of organisms occurred. There is a suggested tendency for all treatments to reduce the actinomycete populations. This is especially true for Br_2.

TABLE 3. Effects of seven test chemicals on the population counts of soil microflora - experiment 3.

Chemicals		Bacteria[1/]	Actino-mycetes[2/]	Fungi[2/]
HF		16.7	9.8	4.6
BF_3		14.7	15.6	4.4
UDMH		13.4	9.4	6.3
Control*		12.2*	29.0*	3.8*
NH_4OH		12.2	15.8	4.4
Br_2		10.6	3.1	4.1
Cl_2		10.3	16.1	3.2
NO_2		8.2	9.5	4.9
L.S.D.	.01	-	-	-
	.05	n.s.d.	n.s.d.	n.s.d.

1/ Ten thousands per gram of oven dry soil
2/ Thousands per gram of oven dry soil

3. Discussion:

Of the 21 chemicals evaluated in these experiments none appeared to have any real deleterious effects on the soil microflora at the concentration of 100 ppm. The possible exceptions are KCN, B_2O_3 and Al_2O_3 which sterilized the soil of actinomycetes. Under conditions where actinomycetes are active in maintaining a balanced microflora

this could lead to undesirable consequences. For example, in a situation where certain actinomycetes may be keeping a fungal plant pathogen in check, removal of the actinomycetes would permit the plant pathogen to be free to attack and kill host plants.

Certain favorable situations are suggested. There is a tendency for $NaNO_2$, HNO_3 and KCN to cause increases in bacterial populations and for $NaNO_2$, $AlCl_3 \cdot 6H_2O$ and $K_2B_4O_7$ to cause increases in actinomycete populations. With the general population increases of these two groups, more than likely, specific bacteria and actinomycetes which are antagonistic to pathogenic organisms would also increase. This could lead to the control of plant and animal diseases which are caused by soil-borne pathogens.

In evaluating these results, it should be remembered that only the surface soil microflora were evaluated and that the chemicals were tested at only one concentration. Also, the chemicals were evaluated separately, not as mixtures. Mixtures of chemicals can have an influence on the soil microflora that differs from the influence each chemical may have when used alone.

D. Soil, Soil Structure and Runoff Water.

1. Introduction:

One of the basic conditions for life on earth is that water be available in uncontaminated, liquid form.

An all-important question at the present time concerns the disposal of industrial wastes as well as wastes from other sources which may contaminate both the soil and water sources. A special case of contamination or pollution, as it relates to human health, concerns the protection of municipal and rural water supplies from radioactive and chemical wastes.

The coming years will bring about an increase in the use of radioactive materials and numerous new chemicals in manufacturing, for the production of electric power, for experimentation in medicine, agriculture and industry.

Radioactive and other fallout materials released into the air are deposited sooner or later on the surface of the earth, including streams and ponds. Many of the radiation and chemical pollutants are non-injurious, and most of that which enters municipal reservoirs or other surface water can be rendered harmless by the application of modern detection and treatment methods. However, not yet known are the longtime, accumulative effects on human beings of the harmful chemicals and radiation that still remain in the water consumed after it has been treated.

Studies indicate that the water which comes from underground sources, such as deep wells, is much less susceptible to contamination from air and surface-borne materials than are the surface and

shallow ground waters. Other research indicates that present distillation methods of purifying water are effective in eliminating most sources of harmful radiation; however, the removal of certain chemical contaminants, such as those being supplied as waste products from missiles, may present a more difficult problem to solve.

Perhaps the same watershed conditions which favor the slow movement of water through the soil into underground storage may prove highly desirable also in facilitating the natural purification of contaminated water. If this is true, watershed management, especially in localities that represent sources of underground recharge, will take on added significance by providing an important safeguard to human health and well being.

Another all-important and basic factor which must be considered and is related directly to the problem of contamination or pollution of waters concerns the soil, especially its texture and mineralogical composition. Soil is a highly complex system and is composed of solid, liquid and gaseous materials. The chemical and physical relationships are affected not only by their respective properties but also by temperature, pressure, and to some extent by light. The mechanical behavior of the soil mass (sand, silt and clay fractions) as affected by these components is referred to as the physical properties of soils.

Clay is involved in almost every reaction in soils. Both chemical and physical properties of soils are controlled to a very large degree by properties of clays, and an understanding of clay properties is essential if a full understanding of physico-chemical interrelations is to be obtained.

It is a known fact that clay is the most active part of the soil, both chemically and physically. In a soil where the clay content is low and sands or silts predominate, most of the pores will be large and continuous so that water and air may move freely. In this case, porosity may be favorable but chemical properties would be unfavorable. However, where clays are more abundant and the chemical properties are more favorable, physical characteristics of the soil may be either good or poor, depending upon the arrangements of the soil particles.

From the standpoint of soil physics and the related work with this project, studies of the clay systems may be made with the objective of arriving at an understanding of "how" the clay properties will affect the whole soil complex. Many chemical properties and reaction of clays can be studied best on separated clay samples, since sand and silt are comparatively inert and act only as carriers or dilutents to the more active clay. Studies of the physical properties of pure clays are of extreme importance in certain phases of clay technology; however, they are of somewhat limited value to soil physics in general. There are two reasons for this: (1) in soil, clay is always mixed with other materials such as sands and silts, both of which strongly affect physical characteristics; (2) physico-

chemical characteristics of clay are greatly changed by the adsorption or combining with certain types of organic compounds which are added to or exist in soil. Because of this, the studies which were conducted under the present project were primarily concerned with clay as it existed in the soil and in combination with the other size-fractions and organic materials.

2. Types of Studies:

The initial studies in soil physics were concerned with determining whether or not the selected materials would be adsorbed to the soil particles (primarily clay) or leached downward through the shallow soil profile (0-4 ft). Additional studies were directed toward the effect of various, selected chemical compounds on soil structure formation and their movement in water from the surface as a result of runoff.

From these considerations it became apparent that to carry out the assignment for soil physics would necessitate a 3-phase program which was outlined as follows:

Phase 1. A leaching study to determine the extent (if any) to which the propellants could be leached downward through the shallow soil profile.

Phase 2. A study concerned with the effect of selected chemical propellants on soil structure formation or destruction. This phase was conducted under laboratory conditions.

Phase 3. A water runoff study which was directed toward determining the relative effect of runoff on the transport of selected chemical propellants from surface soil areas.

In order to be consistent throughout all phases of the study, the 0-8 inch depth of a Lufkin loam soil was used.

3. Phase I. Water Leaching (Percolation) Study:

On reaching the earth's surface all rainwater divides into 3 general categories - runoff, evaporation and/or transpiration (vapor), and percolation downward through the soil profile.

Surface and ground waters are never chemically pure. The most common substances dissolved in ground water are the salts of the common basic radicals. The common bases are Na, K, Ca, Mg, Fe and Al; and the common acid radicals are Cl, SO_4 and CO_3. The rarer bases such as Li, Ba, Cu, Zn, and the acids of A and B may give the water unusual or even toxic properties.

Because of the possibilities of contaminating the shallow ground water through the exhaust or spilling of missile propellants, it was deemed necessary to determine the extent to which the selected chemical materials would percolate with water through a column of soil or

be adsorbed on the clay complex. In the latter case, an absence of the chemical in a test for it in the leachate would indicate possibly 2 things: (1) it was adsorbed on the clay fractions; or (2) it was not leached out by an application of 8 inches of water applied to a previously saturated soil column of Lufkin loam soil.

a. Procedures:

1) As indicated previously, the topsoil of a Lufkin loam was used in the percolation study.

2) Soil columns, 6 1/2 inches in diameter by 42 inches in length, were packed in clear plastic cylinders which were 54 inches in length. Each cylinder was sealed at the bottom with 5/16 inch thick clear plastic and an outlet was opened at the center of the bottom plate for removal of the effluent. A 2-inch thick mixture of 1/2 mortar sand and 1/2 fine gravel (1/4 inch diameter) was placed in the bottom of each cylinder before the addition of the air dry soil. After packing each 42-inch soil column, twelve inches of water were used to saturate the columns. The inch of treated soil was then added and a 1-inch thickness of gravel was placed on the soil surface to prevent puddling when water was added to the column. Eight inches of water were then added for percolation with each treatment. The effluents were retained, and samples were taken daily for analytical purposes to determine whether or not the material under study was moving downward with the percolation of water.

3) Each chemical was mixed thoroughly in the desired concentration (1000 ppm by weight) with the 0-1 inch depth of soil before the addition of the gravel and the 8 inches of water for the test run. All column treatments were made in duplicate only, because time did not permit additional runs for each compound under study. In some cases, the test chemical was added as a water solution just before water was applied for the percolation study.

b. Results:

The results are given in Table 4.

TABLE 4. Results of soil leaching studies with twelve test chemicals.

Treatment No.	Compound	Length of time for study - days	Concentration of effluent in ppm*
1.	Br_2	11 (7/19 - 7/30)	7/19 - 1.14 7/26 - 135.0 7/30 - 64.0

(continued)

(continued)

Treatment No.	Compound	Length of time for study - days	Concentration of effluent in ppm*
2.	$NaNO_2$	41 (6/7 - 7/18)	6/30 - 2.53 7/8 - 6.67 7/12 - 9.50 7/18 - 0.33
3.	HNO_3	11 (7/19 - 7/30)	7/19 - 0.16 7/30 - 8.96
4.	NH_4OH	8 (9/6 - 9/14)	none detected
5.	N_2H_4	8 (9/6 - 9/14)	none detected
6.	Al_2O_3	46 (6/2 - 7/18)	none detected
7.	AlF_3	46 (6/2 - 7/18)	Al - min - 0.0 max - 0.32 F - min - 0.0 max - 0.83
8.	BF_3	8 (9/6 - 9/14)	B - none detected F - 9/6 - 0.65 9/11 - 1.65 9/12 - 2.30 9/13 - 72.60 9/14 - no drainage
9.	B_2O_3	15 (9/6 - 9/21)	B - none detected
10.	$K_2B_4O_7$	8 (9/6 - 9/14)	B - none detected
11.	Li_2O	21 (6/2 - 6/23)	Li - ranged between 10 and 25 at end of run
12.	LiF	51 (6/2 - 7/23)	Li - 6/2 - <25 6/5 - <10 Continues <10 to end of run F - 6/2 - 0.33 6/22 - 0.58 7/23 - 1.02

* Initial input concentration to the soil or percolating water was 1000 ppm by weight, based on 763 grams of oven-dry soil. All values shown were obtained by subtracting control values from experimental values.

Note: References concerning the methods and procedures for chemical analyses will be found in the bibliography.

c. Discussion:

The data as presented indicate that bromine, the nitrite ion, lithium, and fluorine were detected in the effluent in quantities which, over a period of time, may become sizeable if the source remained somewhat at the same level or increased. It is suggested that boron, aluminum and ammonia have been retained within the soil complex.

4. Phase II. Effect of Chemical Compounds on Soil Structure Formation or Deterioration:

It is generally recognized that organic matter in some way has a favorable effect on the formation of aggregates in the soil. An aggregate is a cluster of soil particles held together more or less loosely but with sufficient strength so that it behaves in the soil as a unit. The forces holding particles together are stronger within the aggregates than the forces between aggregates. When a soil is aggregated, the clay particles cause the sand and silt grains to be held together in larger units. This causes an increase in the proportion of larger pores in the soil, thus favoring movement of water and air into and through the soil.

Organic matter is known to act as a binding agent in the stabilization of aggregate clusters. Many investigators have shown that organic matter is not effective as an aggregating agent until it begins to decompose. The fraction of organic matter capable of aggregating soil, in addition to being water soluble, must be decomposed in the soil by the action of micro-organisms. Many researchers have demonstrated that the addition to the soil of rapidly decomposable substances, such as sucrose, and finely ground clover and/or alfalfa, results in a large increase in the number of bacteria and a rapid increase in water-stable aggregates.

It is possible to greatly improve aggregation in fine sandy loam soils by innoculating nonsterile soil in pots with certain cultures of bacteria and fungi, following the addition to the soil of sucrose or ground straw as indicated above. Aggregate formation in the innoculated soil will be much more rapid than in soil receiving similar organic matter treatments without innoculation.

Therefore, since microbiological activity does play an important role in structure formation and stabilization of aggregates, it was necessary to determine the extent of injury or kill which might be expected in soils that were subjected to the selected chemical treatments as outlined in Phase I. If a given compound should be toxic to the soil microorganisms, it was thought that such toxicity would be indicated by a decrease in soil stabilization following a lengthy incubation period.

a. Procedures:

1) There were 18 chemical treatments plus three controls. There were two sets of each of the 21 treatments; one set had no organic matter added while the other set had ground alfalfa (0.5 mm) added at the rate of 15 tons per acre, all treatments were replicated three times.

2) Eight hundred and fifty (850) grams of air-dry soil were placed in 4-inch plastic flower pots. The drainage holes were sealed with heavy tape. The soil used was the 0-8" depth of Lufkin fine sandy loam.

3) The fertility level used was 90 pounds of nitrogen (as ammonium nitrate) and 90 pounds of P_2O_5 (both on a per acre basis).

4) After mixing each soil treatment and placing in the proper container, the required amount of solution and/or salt plus water was added in sufficient quantity to bring the soil to field capacity (150 ml).

5) Water (60 ml) was added at weekly intervals in order to maintain sufficient moisture for decomposition and microbial activity.

6) Thirteen of the treatments were allowed to incubate from May 18, 1961, to November 1, 1961. The other six were not started until July 17, 1961, but were also terminated on November 1, 1961. The temperature ranged between 70° and 80°F.

7) At the end of the incubation period, 2-100 gram samples from each test were used to determine the effect of individual treatments on the weight of stable aggregates which remained after the sample was subjected to a water treatment (this gave 6 samples per test chemical). The wet-sieve method of analysis was used (35). Screen mesh sizes were 5 mm, 2 mm, 1 mm, 0.50 mm, 0.25 mm, and 0.125 mm. The aggregate sizes above 0.5 mm are of main interest to agriculture.

b. Results:

The results of structure formation or destruction, as influenced by the selected chemical compounds, are shown in Tables 5 and 6. Two studies were conducted: (1) in one of the studies there was no organic matter added to the soil before incubation (Table 5), and (2) organic matter was added to the soil in the form of ground alfalfa (0.5 mm) and at a rate of 15 tons per acre (Table 6).

TABLE 5. The aggregate stability of Lufkin fine sandy loam with no added organic matter, as affected by the 18 test chemicals. All values are given in grams.*

Treatment and date started	Size of sieve openings - millimeters					
	5	2	1	0.50	0.25	0.125
HF (7/17)	1.7310	0.6316	0.9119	1.1599	5.7351	14.9099
Cl_2 (7/17)	1.6957	0.9308	0.7753	1.1306	3.7987	20.1864
Br_2 (5/18)	0	1.9291	2.1633	2.0089	4.7860	12.0014
$NaNO_2$ (5/18)	0	0.7125	0.4516	1.2461	3.6431	16.6770
HNO_3 (5/18)	0.7797	0.6987	0.6657	2.0009	7.0266	23.3833
HCl (5/18)	0	1.1515	1.1459	2.3515	5.1638	18.4179
NO_2 (7/17)	0	0.7341	0.8562	2.2484	5.4001	24.4469
KCN (5/19)	0	1.4924	1.6701	3.2893	6.8934	20.2686
NH_4OH (5/19)	0	1.2134	0.8613	1.6964	4.0888	21.4696
N_2H_4 (5/19)	0	1.6228	1.4673	2.8029	9.5228	21.2929
UDMH (7/17)	0	0.7993	0.4296	1.0943	2.4462	19.5851
Al_2O_3 (5/19)	0	0.7560	0.6241	1.2344	3.7006	22.2526
AlF_3 (5/19)	0	0.7881	0.5589	1.3892	3.1566	16.6203
BF_3 (7/17)	0	0.5743	0.5040	0.9254	3.0223	17.1004
B_2O_3 (5/19)	0	0.7277	0.6430	1.1816	4.2283	16.8329
$K_2B_4O_7$ (7/17)	0	0.5725	0.6442	1.5641	5.8385	22.9508
LiO_2 (5/19)	0	0.8750	0.8034	1.5420	6.5286	20.7363
LiF (5/19)	0	1.3033	1.5905	2.8413	7.0596	16.7574
Control 1 (5/18)	0	0.7889	0.7363	2.5159	3.8285	17.8731
Control 2 (5/18)	0	0.4779	0.2700	0.7586	3.5354	19.0267
Control 3 (5/18)	0	0.5191	0.5175	1.2104	5.5815	20.9034

* Values shown are the average weights (on an oven dry basis) of 6 samples per size - range for each soil treatment.

TABLE 6. The aggregate stability of Lufkin fine sandy loam with added organic matter, as affected by the 18 test chemicals. All values are given in grams.*

Treatment and date started	Size of sieve openings - millimeters					
	5	2	1	0.50	0.25	0.125
HF (7/17)	1.0102	2.8832	5.1568	6.4206	14.8263	19.1791
Cl_2 (7/17)	4.4047	8.5921	8.6545	7.4101	7.9651	13.8024
Br_2 (5/18)	3.7333	2.9917	6.5476	7.9182	8.9549	17.0491
$NaNO_2$ (5/18)	6.0859	3.8745	6.0182	7.5511	8.0613	20.0334
HNO_3 (5/18)	5.1797	5.2086	6.9481	6.1451	8.9488	15.6203
HCl (5/18)	4.6548	5.2034	5.2755	6.3714	6.4297	17.8530
NO_2 (7/17)	2.2114	5.5170	7.1130	7.5164	9.9025	17.5599
KCN (5/19)	3.1350	4.8461	6.9182	7.5345	14.8076	19.2602
NH_4OH (5/19)	4.6977	5.5981	7.3151	6.3133	10.7520	16.5574
N_2H_4 (5/19)	0	3.5831	6.3740	7.6007	12.7134	23.3777
UDMH (7/17)	1.0902	4.8861	5.7187	7.5803	11.0458	18.0485
Al_2O_3 (5/19)	5.6943	6.5295	9.2520	6.8598	13.5619	16.1471
AlF_3 (5/19)	9.1778	7.5007	7.2793	7.2446	9.5623	16.7753
BF_3 (7/17)	5.9314	3.8164	3.7401	3.5312	9.3901	20.8168
B_2O_3 (5/19)	9.9190	2.8691	5.0506	7.2095	9.5608	20.1898
$K_2B_4O_7$ (7/17)	3.5142	6.6031	7.5848	9.8285	10.0277	16.9661
Li_2O (5/19)	4.2524	1.5515	2.9121	4.2859	8.1858	20.6259
LiF (5/19)	3.5833	4.6352	6.4669	6.3257	9.8701	19.6631
Control 1 (5/18)	7.8097	4.3068	6.2379	6.3933	8.3182	18.4235
Control 2 (5/18)	4.4328	10.0509	10.4710	8.0790	14.1185	15.8673
Control 3 (5/18)	4.2033	7.6683	7.5966	8.8617	13.2913	19.9209

* Values shown are the average weights (on an oven dry basis) of 6 samples per size - range for each soil treatment.

Values shown in the table are oven-dry weights of soil aggregates which remained on a given-size sieve following a 15 minute water immersion of each sample and washing in water. Two separate samples were obtained from each of the three containers. Thus, each soil treatment had six separate analyses.

c. Discussion:

1) A statistical analysis of this preliminary data was not attempted. However, it may be concluded generally that almost all chemicals used were not effective in reducing appreciably the stability of aggregates by adversely affecting the microbiological population. This agrees with the results obtained in the soil microflora study.

2) The greatest effect on the formation and stabilization of aggregates was brought about by the addition of organic matter. This is in agreement with past studies on the Lufkin fine sandy loam topsoil.

3) Of main interest in agricultural soils are the aggregate sizes 0.50 mm, and especially the 2 and 5 mm sizes. In the study where organic matter was added, HF, Br_2, NO_2, KCN, N_2H_4, and $(CH_3)_2NNH_2$ were perhaps detrimental to the formation of the larger-size aggregates.

4) The trend as outlined in the previous paragraph does not necessarily hold for the soil treatments which did not receive organic matter. The fact that the HF, Cl_2, Br_2, KCN, N_2H_4 and LiF treatments had more of the 2 and 5 mm sizes of aggregates is difficult to explain at this time. It might be postulated that the binding effect in this case could be attributed more to chemical (ionic) bonding than microbiological. However, it should be safe to conclude that in both cases the effects of the chemicals on soil structure, at the levels and period of incubation used, were generally ineffective.

5. Phase III. Effect of Runoff Water on the Transport of Chemical Compounds from Surface Soil Areas:

Precipitation falling to the earth's surface is either retained where it falls, pases through the soil surface by infiltration and then percolates downward, or finds its way into the surface channel system of the basin, whence it becomes surface runoff.

A considerable portion of the rain at the beginning of a storm is stored on the vegetal cover as interception and in surface puddles as depression storage. However, as rain continues, the soil surface becomes covered with a film of water which begins to flow downslope toward an established channel.

The rate of erosion in any storm depends on the force with which raindrops stir-up soil and the amount as well as the speed of the

runoff water. Other factors affecting erosion include the kind and amount of surface cover, type and texture of soil, steepness and length of slope, and antecedent soil moisture.

Structure conditions of the soil surface affect greatly the infiltration of water and diffusion of air into the soil. Whenever the surface layer becomes puddled by a hard rain or compacted by heavy machinery, the danger of runoff and erosion increases and the infiltration and storage of water within the root zone of plants decrease.

This phase of the overall fallout and/or contamination problem was considered to be an important aspect because of the large amounts of surface waters and loose topsoil which are transported from watersheds to confined channels or streams after each high intensity rain of several inches. There are 2 parts of this particular problem which warrant consideration at this time. First, since excess water will be present, many of the more soluble compounds can and probably will be transported to a great extent by this vehicle of runoff water. Second, those compounds which are not soluble in water, may be adsorbed on the clay complex of the loose surface soil or clay particles in suspension which would then make it possible for them to be transported by the runoff of surface waters. In either case, movement would be rapid and contamination of both stream and reservoir waters could be the end result.

It becomes obvious, therefore, that the vehicle or mode of transportation will be an important part of the study. Whether movement occurs in a solution form or whether it is brought about by being adsorbed on the clay and then transported (or both) must be determined. The initial study conducted and to be reported herein concerned the former question only because it could be attacked much more quickly and less expensively at this time than the latter. Moreover, it should be emphasized that the study, although somewhat preliminary in nature, did indicate that water was an important transport medium for some of the compounds used.

a. Procedures:

1) The 0-8 inch depth of Lufkin fine sandy loam soil was used in the study.

2) The plot area consisted of 4 plots, each of which was 1 foot wide and 18 feet in length. The plot area was made of wood and braced under the bottom by A-frames which provided a slope of 4 percent. Individual plots were separated by 1 x 4 inch wood-strips, and soil was placed to a depth of 3 inches in each plot. Each chemical was run in duplicate - one beside the other. Fresh soil was used with each chemical to prevent contamination between chemicals.

3) After the 4 plots were filled with 3 inches of fresh soil and leveled, fine mist sprays, which were spaced 15 inches apart

and at a height of 2 feet above each plot, were turned on and permitted to apply water until the soil mass was saturated and somewhat puddled on the surface. The plots were then allowed to drain by gravity overnight and until 9:00 a.m. the next day. The chemical treatments were then applied. Such surface and soil moisture conditions provided maximum runoff potential for each plot.

4) Chemical treatments were applied in a square 12 x 12 inches by 1/4 inch thick at the head of the runoff plot.

5) After the test chemical, plus dry soil, had been placed at the head of the runoff plot, water was turned on through the spray system at an intensity of approximately 12 inches per hour. At this time about 3 ml of red dye was applied to the soil surface at the bottom edge of the treated soil and followed visually to the exit end of the plot. The initial water sample was taken when the red dye reached the discharge end of the plot. Samples were taken at 3-minute intervals for a period of 18 minutes, giving 7 samples per plot run. The samples were returned to the laboratory, allowed to settle for 24 hours, and then filtered to remove any of the colloidal clay which remained in suspension at that time. The samples were then analyzed for the specific chemical or ionic components.

b. Results: The results are given in Table 7.

TABLE 7. The concentration of specific test compounds or ionic components (in ppm) in samples collected in water runoff studies.

Chemical* Compound	Time of Sampling - Minutes						
	0	3	6	9	12	15	18
LiF	>3.0	>3.0	2.4	2.1	1.3	1.4	1.1
$NaNO_2$	2.9	1.9	1.6	0.8	0.8	0.7	0.8
AlF_3	1.1	0.9	1.3	1.5	1.5	2.1	2.1
HF	>3.0	>3.0	>3.0	>3.0	>3.0	2.5	1.9
NH_4OH	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N_2H_4	1.9	0.0	0.1	0.0	0.0	0.1	0.0
BF_3	1.9	1.8	2.3	1.6	1.3	1.1	1.0

* The chemical or ion tested for is underlined.

c. Discussion:

1) The method used for the runoff studies is acceptable and can be used for future laboratory studies of this kind.

2) The data presented could be the result of or indicate several things: (a) the chemicals applied are being transported in water at a much slower rate than usually considered; (b) they could be adsorbed on the colloidal clay complex and removed partially or almost entirely during the filtering or decanting processes; (c) they could remain in the treated soil and not be transported in the water stream at all: (d) the sampling time was not extended sufficiently long; (e) or the method of applying chemicals to the soil was inadequate.

3) The ammonia data are valid because of the rapidity by which ammonia is adsorbed on clay. Therefore, assuming that the other limited data also are valid, the amount of contamination (based on concentrations obtained) from such runoff would appear to be small. It is even postulated that lower concentrations would occur from soils with higher clay content than the Lufkin loam.

E. Plant Growth and Development

1. Seed Germination:

a. Methods and materials:

1) Chemicals and their use.

All chemicals were prepared as outlined (III-B-4) and tested with no pH adjustment. In addition, the following chemicals were also tested after neutralization with KOH or HCl to pH 6.5: UDMH, N_2H_4, Cl_2, Br_2 and BF_3. A second group, $K_2B_4O_7$ and Li_2O were also tested after being neutralized to pH 7.0.

2) Germination studies were done in a controlled temperature cabinet at 89 $\pm$ 1°F under a 70 $\pm$ 3% relative humidity. Six inch petri dishes containing a Kim Pac germination pad covered with filter paper were used as seed containers. These dishes with pads were sterilized in an autoclave for 15 minutes, under 15 lbs pressure, at 121°C before use in the experiments.

3) Five test seeds were used in these experiments (III-B-1). The seeds were selected free from visible mechanical and insect damage, sterilized in a 5% chlorox solution for 30 seconds, rinsed 6 times and soaked in the appropriate test solution for 2 hours. They were then removed and placed in petri dishes. Twenty-five seeds were placed in each petri dish and all experiments were replicated to give a total of 50 seed per test solution.

Seeds were watered with 25 ml of a test solution containing 1000 ppm of the test chemical and the dishes were then placed in the germination chamber. The seeds were left for 2 to 3 days with twice daily checks on the number germinated.

When control roots were 1 1/2 inches long, the experiment was terminated.

4) The germination studies were carried out in five different experiments. Each experimental setup utilized a water control plus five test compounds for each of the five seed types.

b. Results:

The rate of germination was determined from visual counts of seed germinated at 8-hour intervals throughout the course of the experiment. The results are presented graphically in Figures 1-6. The five experiments conducted with each plant were the same. Thus, Table 8 has been developed as a key to the results shown in Figures 1-6. Due to space limitations, we have presented only those curves which gave a 10 percent or greater deviation from the control curve. This was arbitrarily selected as being of possible significance. However, data from these figures has not been subjected to statistical analysis.

The total percent germination (a seed was considered germinated when the radical was 2 mm long - either inside or outside the seed coat), the average growth in length of the young seedlings (includes root and shoot development) and the extremes of growth in length are presented in Tables 9-13. All growth in length is measured in mm. The average length was obtained from germinated seed only.

2. Seedling Studies:

a. Methods and materials:

All plants were started in the greenhouse. Squash, soybean and cotton were planted from seed sixteen days before use while cowpea and corn were planted nine days before use. Six seed of each species were planted in each of eight pots. When plants were 8-12 days old, the pots were thinned leaving the two most uniform plants per pot. Four pots (eight plants) of each species were used in the control and four in the experimental chambers.

All plants were grown in a peat-perlite mix. Pots were sub-irrigated three times a week with a quarter strength Hoagland's nutrient solution before use in the experimental chambers.

The growing medium was moist when the plants were transferred to the test chambers. After the experimental test period, the plants were returned to the greenhouse for a ten-day observation

TABLE 8. A key to the results presented graphically in Figure 1-6.

Key lines	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
————	H_2O	H_2O	H_2O	H_2O	H_2O
- - - - - -	AlF_3 (6.1)*	KCN (10.5)*	UDMH (10.2)*	NO_2 (2.0)*	N_2H_4 (6.5)*
-•-•-•-•-•-	Al_2O_3 (7.2)	N_2H_4 (9.8)	NH_4ClO_4 (5.8)	Li_2O (7.0)	Cl_2 (6.5)
x-x-x-x-x-x	B_2O_3 (6.8)	NH_4OH (10.6)	BF_3 (2.3)	Li_2O (12.3)	Br_2 (6.5)
• • • • • •	LiF (7.5)	HCl (1.8)	Cl_2 (2.6)	$K_2B_4O_7$ (7.0)	UDMH (6.5)
x x x x x x	$NaNO_2$	HNO_3 (1.9)	Br_2 (3.1)	$K_2B_4O_7$ (9.0)	BF_3 (6.5)

* The pH of the water solution containing 1000 ppm test chemical.

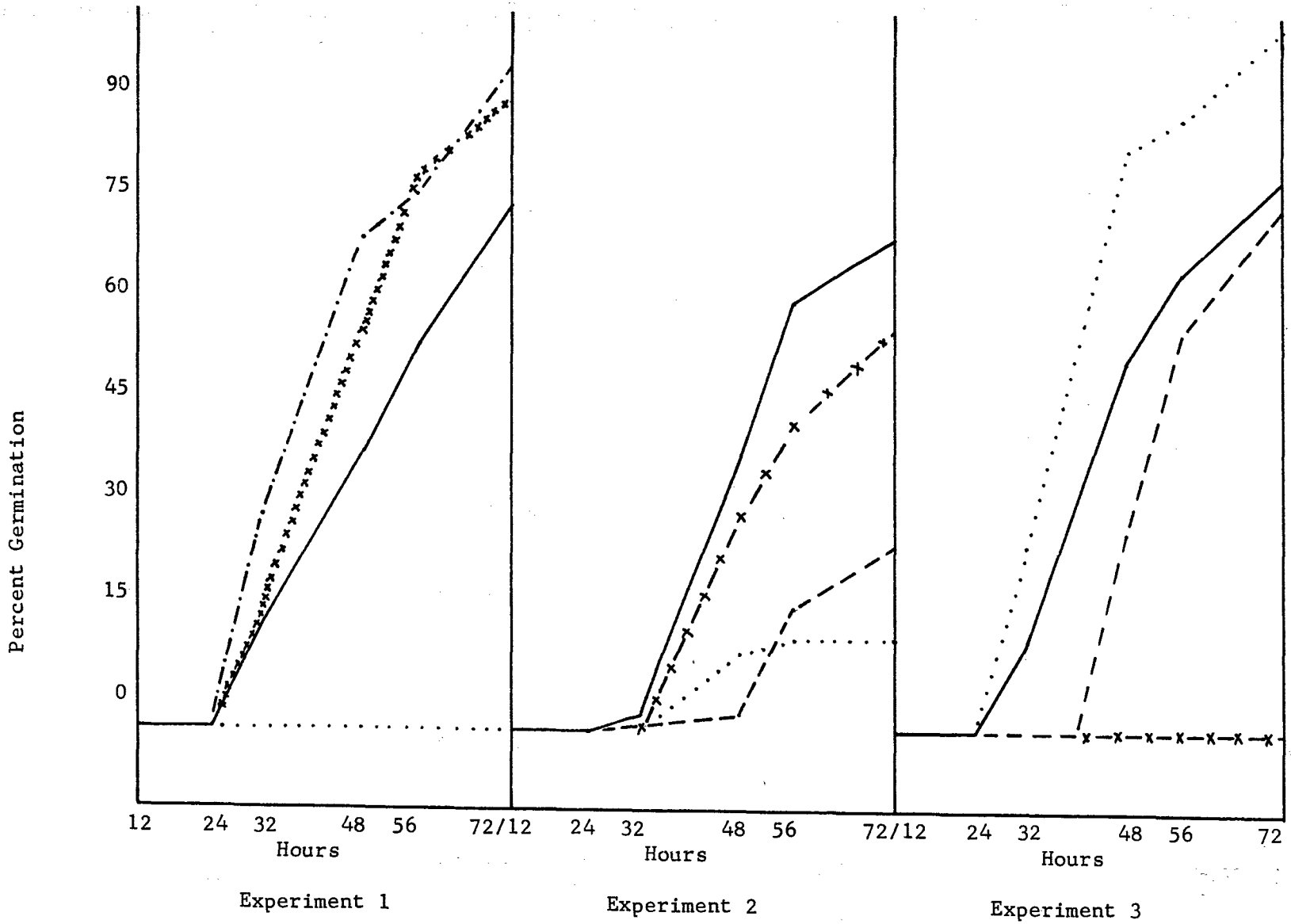

FIGURE 1. Rate of germination in squash treated at 1000 ppm with 15 test solutions. Only those chemicals which gave more than 10 percent variation in germination (from control) were plotted.

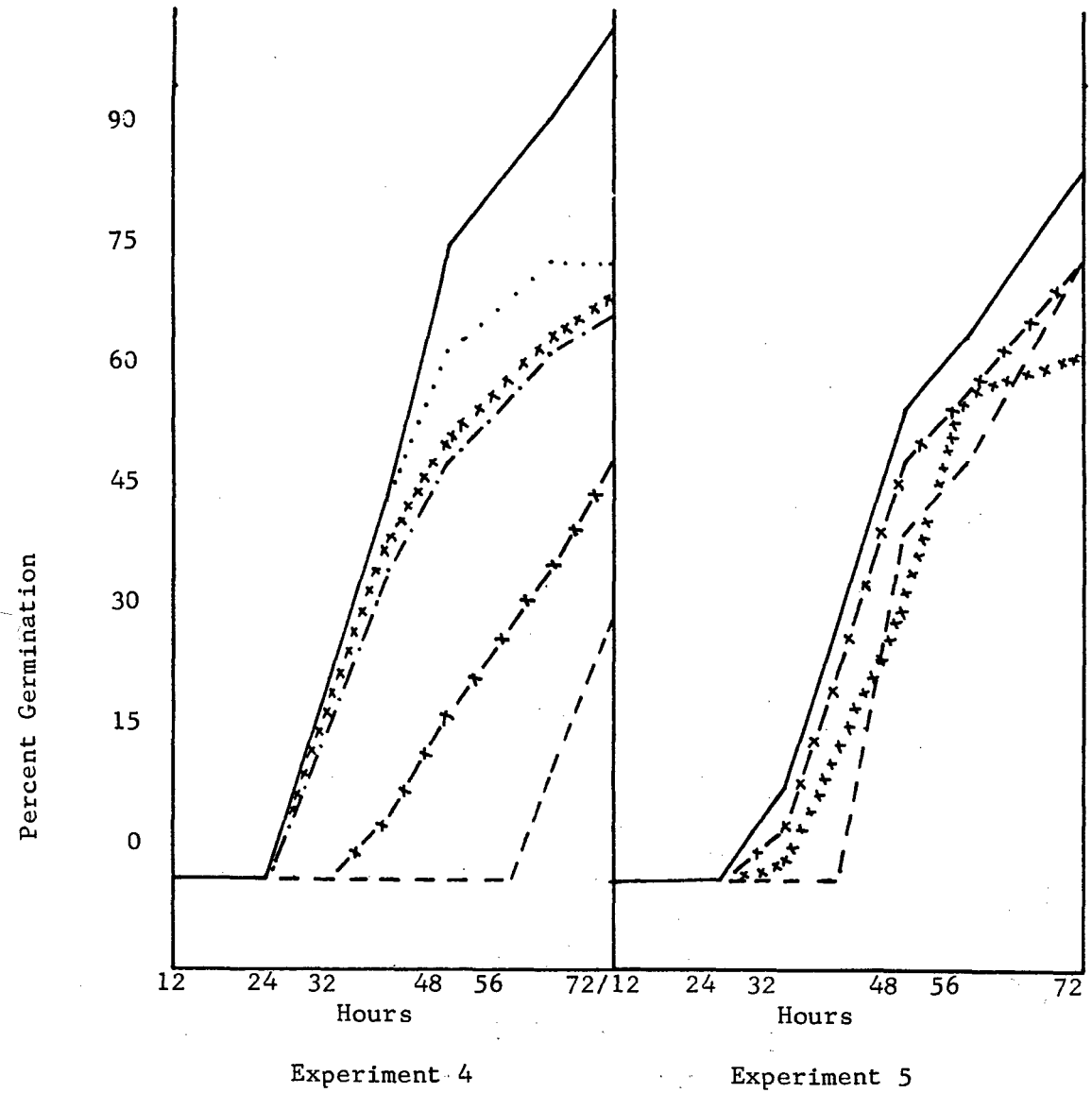

FIGURE 2. Rate of germination in squash treated at 1000 ppm with 10 test solutions. Only those chemicals which gave more than a 10 percent variation in germination (from control) were plotted.

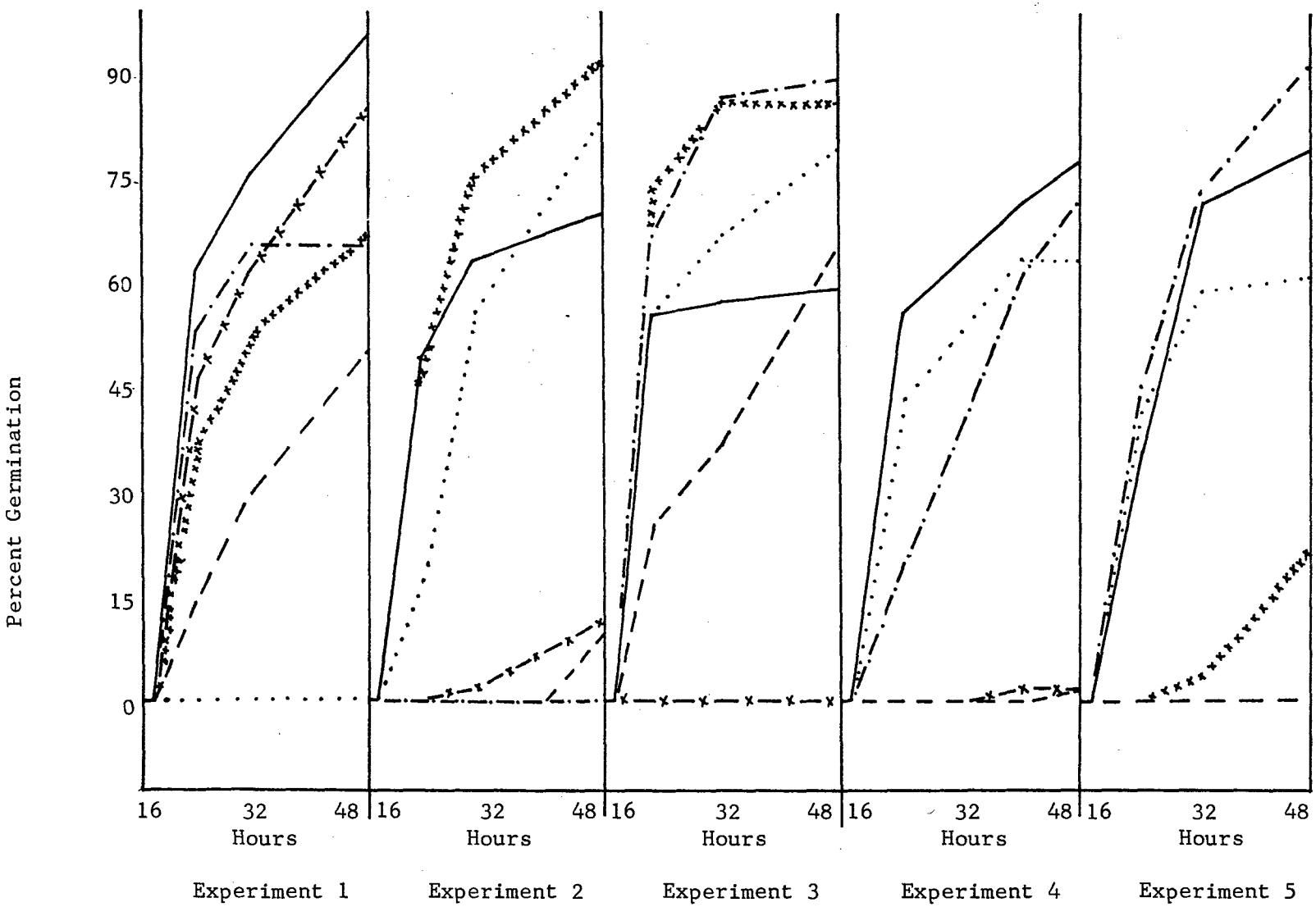

FIGURE 3. Rate of germination in soybean treated at 1000 ppm with 25 test solutions. Only those chemicals which gave more than a 10 percent variation in germination (from control) were plotted.

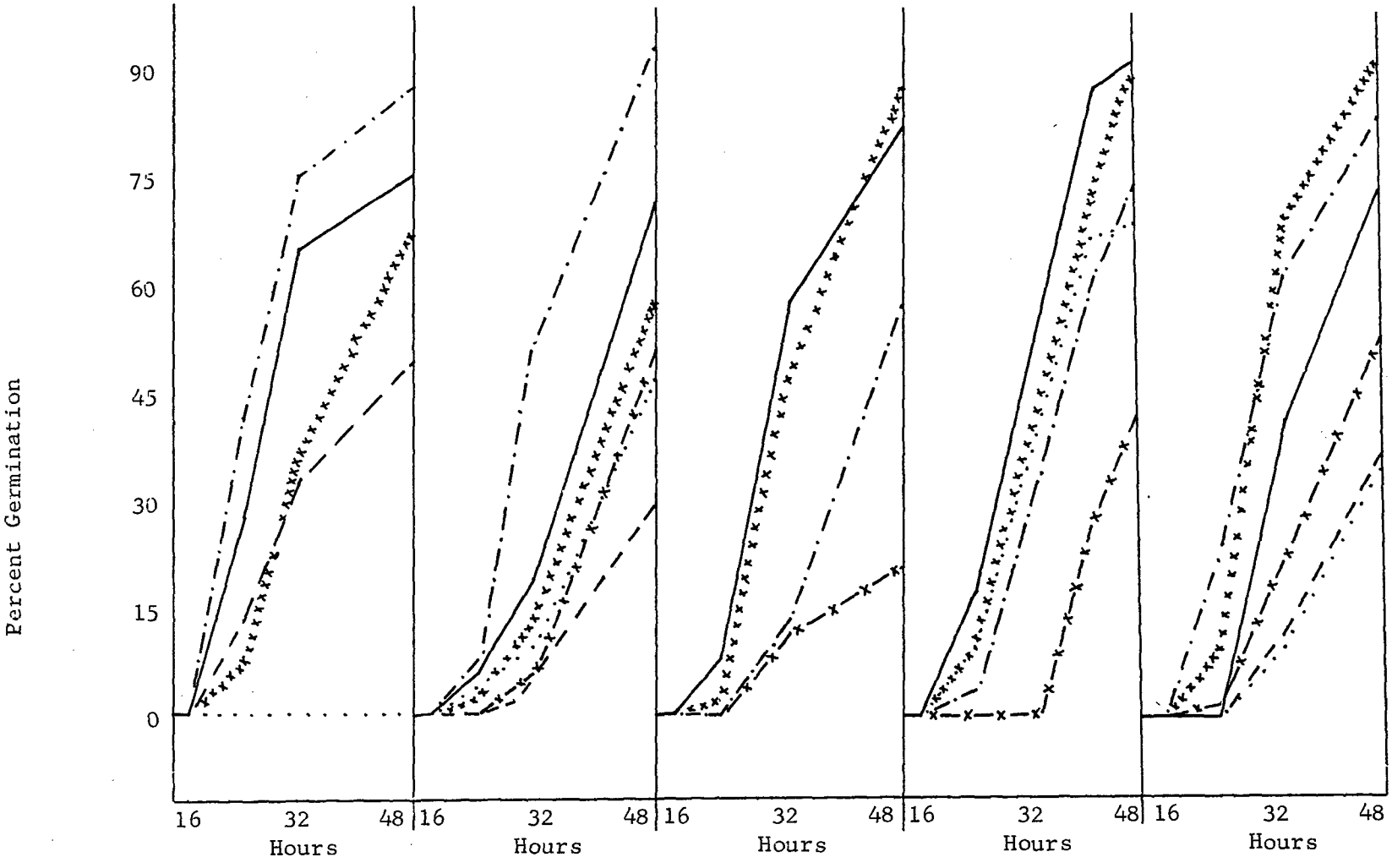

FIGURE 4. Rate of germination in cotton treated at 1000 ppm with 25 test solutions. Only those chemicals which gave more than a 10 percent variation in germination (from control) were plotted.

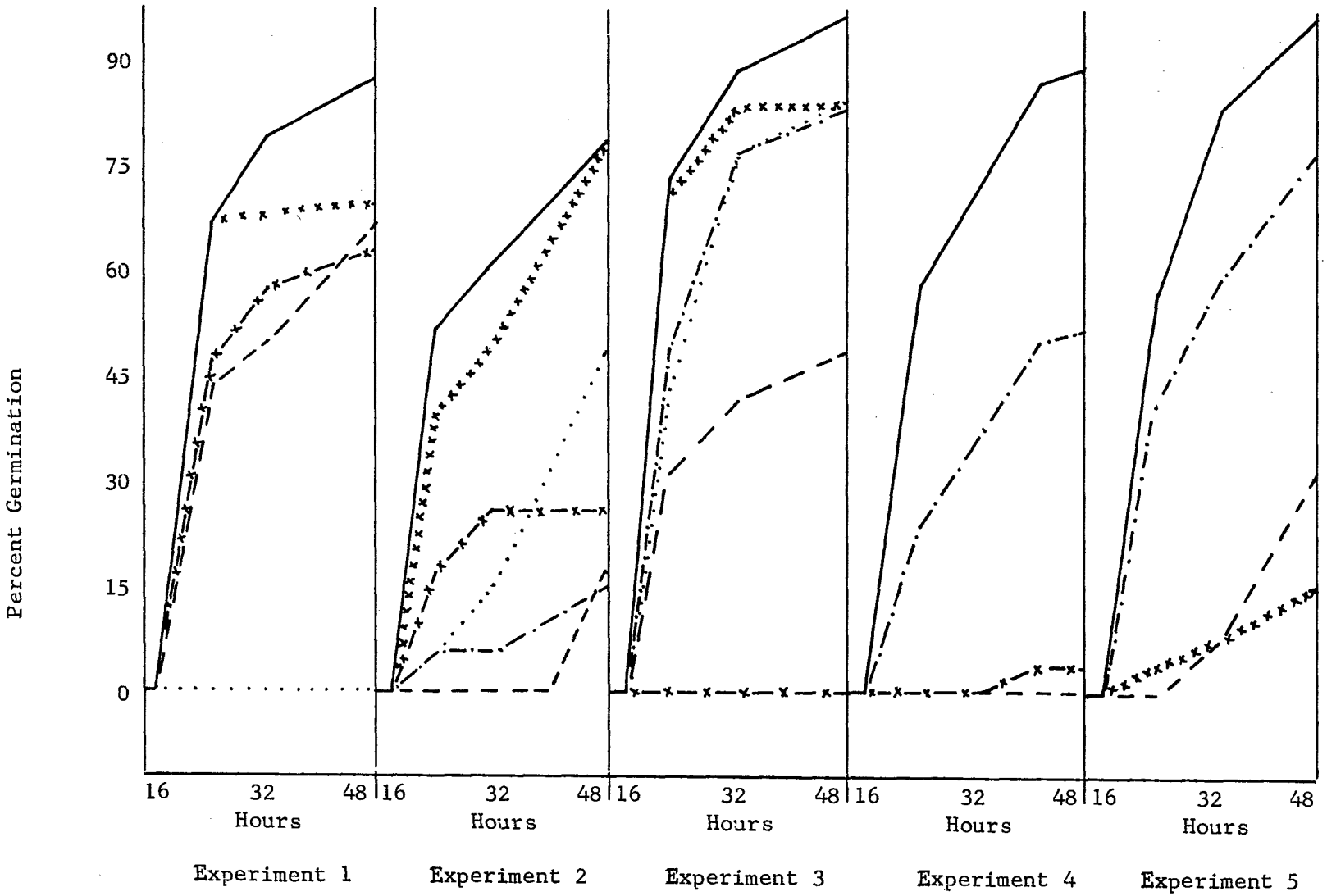

FIGURE 5. Rate of germination in cowpea treated at 1000 ppm with 25 test solutions. Only those chemicals which gave more than a 10 percent variation in germination (from control) were plotted.

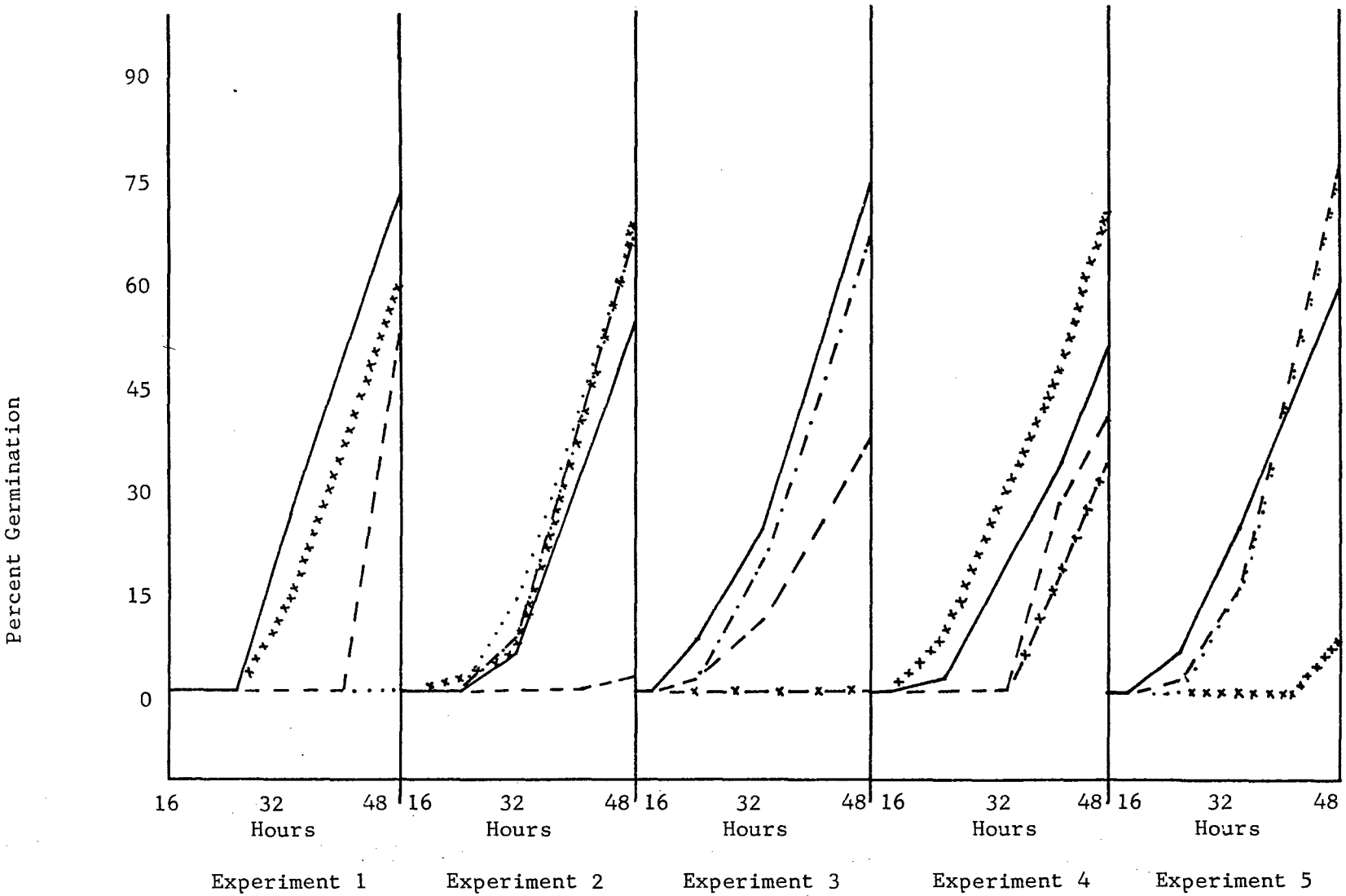

FIGURE 6. Rate of germination in corn treated at 1000 ppm with 25 test solutions. Only those chemicals which gave more than a 10 percent variation in germination (from control) were plotted.

TABLE 9. Germination and growth of squash seed treated at 1000 ppm with 25 test solutions.

Expt. No.	Treatment	Solution pH	Percent germination	Average growth in length (mm)	Extremes of growth in length (mm)
1	H_2O		68	31.2	2-80
	AlF_3	6.1	76	15.0	2-32
	Al_2O_3	7.2	86	43.6	2-93
	B_2O_3	6.8	70	7.8	2-14
	LiF	7.5	0	-	-
	$NaNO_2$		82	33.8	4-73
2	H_2O		64	32.1	3-76
	KCN	10.5	24	11.0	3-21
	N_2H_4	9.8	66	12.6	2-30
	NH_4OH	10.6	52	20.8	5-43
	HCl	1.8	12	2.8	2-3
	HNO_3	1.9	56	5.8	2-12
3	H_2O		72	37.7	4-88
	UDMH	10.2	68	10.2	2-24
	NH_4ClO_4	5.8	80	29.2	2-65
	BF_3	2.3	0	-	-
	Cl_2	2.6	92	34.6	2-67
	Br_2	3.1	74	45.4	5-85
4	H_2O		96	41.1	2-97
	NO_2	2.0	28	2.5	2-5
	Li_2O	7.0	62	3.2	2-5
	Li_2O	12.3	46	2.0	2-3
	$K_2B_4O_7$	7.0	68	13.4	4-22
	$K_2B_4O_7$	9.0	64	14.1	2-28
5	H_2O		78	37.9	2-84
	N_2H_4	6.5	68	4.9	2-10
	Cl_2	6.5	76	41.5	2-78
	Br_2	6.5	68	39.0	5-96
	UDMH	6.5	70	24.1	5-55
	BF_3	6.5	58	12.5	2-26

TABLE 10. Germination and growth of soybean seed treated at 1000 ppm with 25 test solutions.

Expt. No.	Treatment	Solution pH	Percent germination	Average growth in length (mm)	Extremes of growth in length (mm)
1	H_2O		96	29.2	3-57
	AlF_3	6.1	50	9.8	3-24
	Al_2O_3	7.2	66	24.2	6-48
	B_2O_3	6.8	86	12.9	4-28
	LiF	7.5	0	-	-
	$NaNO_2$		68	26.4	5-50
2	H_2O		70	33.7	5-59
	KCN	10.5	10	10.2	6-17
	N_2H_4	9.8	0	-	-
	NH_4OH	10.6	12	10.5	3-19
	HCl	1.8	84	10.7	3-20
	HNO_3	1.9	92	15.0	5-29
3	H_2O		60	35.9	10-70
	UDMH	10.2	66	10.8	3-23
	NH_4ClO_4	5.8	90	29.1	4-50
	BF_3	2.3	0	-	-
	Cl_2	2.6	80	25.2	4-46
	Br_2	3.1	86	32.6	10-56
4	H_2O		78	30.2	5-65
	NO_2	2.0	2	5.0	5-5
	Li_2O	7.0	72	10.0	3-16
	Li_2O	12.3	2	6.0	6-6
	$K_2B_4O_7$	7.0	64	12.9	3-32
	$K_2B_4O_7$	9.0	-	-	-
5	H_2O		80	21.5	7-50
	N_2H_4	6.5	0	-	-
	Cl_2	6.5	92	27.6	6-52
	Br_2	6.5	76	25.6	5-47
	UDMH	6.5	62	20.7	6-38
	BF_3	6.5	22	6.0	4-8

TABLE 11. Germination and growth of cotton seed treated at 1000 ppm with 25 test solutions.

Expt. No.	Treatment	Solution pH	Percent germination	Average growth in length (mm)	Extremes of growth in length (mm)
1	H_2O		76	45.7	19-60
	AlF_3	6.1	50	23.2	9-40
	Al_2O_3	7.2	88	42.0	10-65
	B_2O_3	6.8	80	22.1	5-32
	LiF	7.5	4	3.0	2-4
	$NaNO_2$		68	24.6	6-50
2	H_2O		72	33.6	9-73
	KCN	10.5	30	23.6	11-34
	N_2H_4	9.8	94	33.2	11-52
	NH_4OH	10.6	52	18.3	11-35
	HCl	1.8	48	14.0	9-21
	HNO_3	1.9	58	16.6	9-28
3	H_2O		82	37.4	15-78
	UDMH	10.2	76	17.5	6-25
	NH_4ClO_4	5.8	58	22.1	5-37
	BF_3	2.3	22	7.6	6-10
	Cl_2	2.6	88	36.4	12-65
	Br_2	3.1	88	35.7	7-58
4	H_2O		92	41.7	9-73
	NO_2	2.0	88	15.2	8-25
	Li_2O	7.0	74	22.9	7-40
	Li_2O	12.3	42	15.7	5-28
	$K_2B_4O_7$	7.0	70	25.8	7-38
	$K_2B_4O_7$	9.0	90	26.6	7-40
5	H_2O		74	28.1	7-56
	N_2H_4	6.5	38	17.5	8-30
	Cl_2	6.5	84	41.1	12-67
	Br_2	6.5	54	29.0	4-65
	UDMH	6.5	36	21.9	7-36
	BF_3	6.5	92	13.8	7-30

TABLE 12. Germination and growth of cowpea seed treated at 1000 ppm with 25 test solutions.

Expt. No.	Treatment	Solution pH	Percent germination	Average growth in length (mm)	Extremes of growth in length (mm)
1	H_2O		88	31.3	9-51
	AlF_3	6.1	68	23.8	6-43
	Al_2O_3	7.2	80	22.1	6-40
	B_2O_3	6.8	64	15.1	3-30
	LiF	7.5	0	-	-
	$NaNO_2$		70	26.1	10-50
2	H_2O		80	30.4	4-53
	KCN	10.5	18	13.1	5-23
	N_2H_4	9.8	16	6.8	3-11
	NH_4OH	10.6	26	18.5	13-27
	HCl	1.8	50	10.7	4-25
	HNO_3	1.9	80	17.4	3-30
3	H_2O		98	24.1	3-53
	UDMH	10.2	50	15.1	4-28
	NH_4ClO_4	5.8	84	21.8	4-45
	BF_3	2.3	0	-	-
	Cl_2	2.6	86	26.5	8-60
	Br_2	3.1	84	28.5	6-54
4	H_2O		90	28.3	3-65
	NO_2	2.0	0	-	-
	Li_2O	7.0	52	10.5	3-15
	Li_2O	12.3	4	12.0	8-16
	$K_2B_4O_7$	7.0	88	28.0	7-46
	$K_2B_4O_7$	9.0	90	24.6	6-46
5	H_2O		98	23.7	2-50
	N_2H_4	6.5	32	7.9	4-11
	Cl_2	6.5	78	25.9	9-52
	Br_2	6.5	98	23.4	6-47
	UDMH	6.5	88	26.1	4-48
	BF_3	6.5	16	15.1	5-29

TABLE 13. Germination and growth of corn seed treated at 1000 ppm with 25 test solutions.

Expt. No.	Treatment	Solution pH	Percent germination	Average growth in length (mm)	Extremes of growth in length (mm)
1	H_2O		72	21.9	3-60
	AlF_3	6.1	52	7.3	2-17
	Al_2O_3	7.2	80	20.1	2-74
	B_2O_3	6.8	76	20.2	2-41
	LiF	7.5	0	-	-
	$NaNO_2$		60	13.8	2-38
2	H_2O		54	20.7	3-58
	KCN	10.5	2	2.0	2-2
	N_2H_4	9.8	66	19.0	2-40
	NH_4OH	10.6	58	12.0	2-34
	HCl	1.8	66	13.1	2-52
	HNO_3	1.9	68	13.4	2-50
3	H_2O		74	15.2	2-56
	UDMH	10.2	38	9.2	2-18
	NH_4ClO_4	5.8	66	14.7	2-36
	BF_3	2.3	0	-	-
	Cl_2	2.6	66	10.0	2-35
	Br_2	3.1	74	25.2	3-74
4	H_2O		50	17.4	3-44
	NO_2	2.0	40	7.1	2-21
	Li_2O	7.0	46	6.5	2-17
	Li_2O	12.3	34	4.9	2-13
	$K_2B_4O_7$	7.0	44	11.6	2-31
	$K_2B_4O_7$	9.0	70	14.7	2-36
5	H_2O		60	18.5	2-82
	N_2H_4	6.5	76	10.0	2-21
	Cl_2	6.5	60	13.2	2-42
	Br_2	6.5	60	18.8	2-54
	UDMH	6.5	76	14.1	2-33
	BF_3	6.5	8	20.8	2-2

period. During this period they were watered three times a week with the quarter strength nutrient solution.

A series of six experiments was run in the four growth chambers to study the effects of the test chemicals, when added to the soil, on plant growth and development. In each experiment, chamber one was used as the control chamber and a test chemical was used in the other three chambers. Thus, 18 chemicals were tested in this fashion. Each chamber contained 4 pots (8 plants) of each of the five species used. The plants were watered with 18 liters of the nutrient solution plus sufficient test chemical to give 100 ppm in the nutrient solution. The test plants were grown in this solution for one week in the test chambers. Plants were observed before addition of the nutrient solution and several times daily throughout the course of the experimental treatment. Daily observations were recorded following the injury index outlined in Figure 7. Plant height, leaf number and cotyledon number (where appropriate) were recorded at the beginning and end of the experimental treatment.

A single experiment was run using fumigation techniques with three test gases. Plants were placed in the test chambers, fumigated at approximately 100 ppm for three hours, removed and placed in the greenhouse for observation (one pot - 2 plants - of each species was removed from each treatment one hour after fumigation started). Continuous observations were recorded during the three hour fumigation and twice daily observations while in the greenhouse. No plant measurements were attempted with these studies. The following conditions were maintained during the course of the experiment: temperature - 84°F; humidity - 55%; soil - moist; lighting - full; average gas concentration - NH_3 (98.7 ppm), NO_2 (81.2 ppm), Cl_2 (62 ppm).

b. Fumigation:

1) Measurement of air flow:

Air flow is measured with the specially designed orifice plates on the outlet tubes. Each outlet has a static pressure pick-up tube just below the orifice plate. These tubes are carried through the chamber walls to a central static pressure pick-up board. In addition, a chamber static pressure pick-up is brought from each chamber to this board. Thus, each chamber has a static pressure pick-up on each outlet tube plus a chamber pick-up. The air flow through each orifice is obtained by measuring the static pressure on either side of the outlet orifice and inserting the pressure differential, as measured with a water manometer, into the following equation:

$$CFM = k \sqrt{p}$$

Where CFM is cubic feet per minute of air flow, p is the pressure differential in inches of water and k is a constant which

includes the following variables: duct diameter, density of air at a given temperature, the delivery coefficient, and orifice diameter. The constant (k) was equal to 15 with the orifice plates used in these experiments. Chamber air flow is obtained by adding the air flow from the two chamber outlet tubes.

Air flow in the experimental chambers for the fumigation studies was about 900 liters per minute.

2) Measurement of gas flow for a chamber concentration of 100 ppm (volume):

a) Calibration of capillary flow tubes. The test gas was metered into the experimental chamber by use of a calibrated capillary flow tube. The flow tubes used in the experiment were our #4 flow tubes (129 mm long, 5 mm OD and 0.5 mm ID). The tubes were calibrated by using dry N_2. The nitrogen flowed: through a Y tube, one end connected to an oil manometer (calibrated in 1/16 inches) and the other to the capillary tube; through the capillary tube into one end of a Y tube (connected by the 2nd arm to the other side of the oil manometer); then, through a calibrated soap film flow meter. Flow rates were recorded for a series of manometer readings and flow in cc/min. vs pressure in 1/16 inches was plotted on standard graph paper. The results plotted as a straight line and the graph was used in determining N_2 flow rates.

b) Calculation of gas flow using the above capillary flow tube.

Flow rates of gases through capillary tubes are a function of gas density or specific gravity. Standard references relate specific gravities of most gases to air (equal 1) at standard pressure and temperature (or temperature as noted). When gases other than air flow through capillary tubes, a correction factor can be used to calculate the flow rates of these gases. This correction factor is obtained using the formula:

$$\text{C.F.} = \sqrt{1/P}$$

where C.F. is the correction factor and P is the specific gravity of gas, other than air (based on the specific gravity of air as 1). The following equation is used to calculate the theoretical N_2 flow based on a desired test gas flow:

$$\text{N_2 Flow (cc/min)} = \frac{\text{test gas flow} \times 1.017 \text{ (C.F. of } N_2 \text{ to air)}}{\sqrt{1/P} \text{ (C.F. of test gas to air)}}$$

The specific gravity value should be for 70°F but many

values are given for 0°C only. The difference between 0°C and 70°F is slight (for our purposes) and where no value for 70°F has been found, we have used the 0°C value.

A chamber concentration of 100 ppm gas with an air flow of 900 liters per minute requires 90 cc of gas per minute. To obtain this flow through the calibrated capillary tubes, the above equation is used to obtain the N_2 flow. From the graph of N_2 flow versus pressure the pressure required to meter 90 cc/min of the test gas is obtained.

c) Method of injecting gas into experimental chamber.

The gas injection apparatus is enclosed within a ventilated chamber beneath the growth chambers. The test gas is injected into a glass tubing apparatus with tygon connections. The gas passes through a Y tube, one arm going to the oil manometer, the other to the capillary flow tube. The gas then passes through the flow tube, mixes with a N_2 sweep gas which carries it to a Y tube connector, one arm going to one inlet duct, the other arm to the second inlet duct in the experimental chamber. These lines enter the chamber and release the gas-N_2 mixture into the air stream from 4 to 6 inches below the point where the air enters the chamber proper. This assures good mixing of the gas in the air stream.

The N_2 sweep gas tubing is connected to the far side of the oil manometer and to the capillary flow tube. The sweep gas is used as an initial dilutant for the test gas and to obtain a more rapid gas flow into the chambers.

The gas flow was adjusted to five the calculated pressure reading on the oil manometer.

d) Actual test gas flow into the test chambers varies with tank pressure and room temperature. In this experiment a manual needle valve was used with each tank of gas. This causes a reduction in flow when tank pressure is reduced. This was a source of error with the gases used and was compensated for by readjusting the needle valve during the experimental period.

c. Results:

1) Soil drench studies:

There were essentially no variations in plant size, leaf or cotyledon numbers in the soil treatment studies. There was visual injury symptoms which were rated according to Figure 7. These symptoms are summarized in Table 14. Only

Symptom	0	1 = Slight	2 = Moderate	3 = Severe
A. Growth	Same as control	Less than control	25 percent less than control	50 percent less than control
B. Epinasty	None	Apparent in any plant part	Approximately 1/3-2/3 of the whole plant or plant part	All or nearly all of the whole plant or plant part
C. Chlorosis	None	Apparent in any plant part	Approximately 1/3-2/3 of the whole plant or plant part	All or nearly all of the whole plant or plant part
D. Necrosis	None	Apparent in any plant part	Approximately 1/3-2/3 of the whole plant or plant part	All of nearly all of the whole plant or plant part
E. Abscission	None	At least one leaf other than cotyledons	At least 1/2 of original leaves other than cotyledons	Complete abscission of all leaves
F. Flowers	Same as control	Any of above conditions list as 1-A, 1-B, 1-C, etc.	Any of above conditions list as 2-A, 2-B, 2-C, etc.	Any of above conditions list as 3-A, 3-B, 3-C, etc.
G. Cotyledons	Same as control	Any of above conditions list as 1-A, 1-B, 1-C, etc.	Any of above conditions list as 2-A, 2-B, 2-C, etc.	Any of above conditions list as 3-A, 3-B, 3-C, etc.

FIGURE 7. Symptoms of plant injury and the numerical index used to rate the degree of severity of injury.

TABLE 14. Plant injury after growth in 100 ppm of test chemical added to the nutrient solution.

Chemical	Plant	Symptoms*	Symptom Location
AlF_3	Squash	Growth - 3	Leaf blade
LiF	Squash	Chlorosis - 2; Necrosis - 1	Leaf margin
	Soybean	Chlorosis - 3; Necrosis - 2	Interveinal
BF_3	Squash	Chlorosis - 1; Necrosis - 1	Leaf margin
	Corn	Chlorosis - 1	Leaf blade
BeF	Squash	Chlorosis - 3; Necrosis - 1	Leaf margin
LiO	Squash	Chlorosis - 2-3; Necrosis - 1	Leaf margin, interveinal
$K_2B_4O_7$	Squash	Chlorosis - 1; Necrosis - 1	Leaf margin

* As outlined in Figure 7.

the plants injured and the chemical causing injury are listed in this table.

2) Fumigation studies:

The initial effects of the three test gases were noted within 5-10 minutes after the fumigation started. The NO_2 and Cl_2 produced a rapid browning necrosis with loss of turgor in all plants. The leaves were affected in the following order: young mature leaves, mature leaves, and then young leaves. In most cases the young leaves were not killed. When death did result, it was usually the death of stem tissue which secondarily caused death of the very young leaves. Complete death was not noted until after the plants were placed in the greenhouse.

The NH_3 fumigation produced effects similar to the above two gases but the chlorophyll was not destroyed. Dead plant tissue was a dried greenish color.

There were essentially no changes in the severity of plant injury after a 20-hour period in the greenhouse. On the basis of close observation, the following order of injury would be appropriate for each test gas on each plant:

Squash - $Cl_2 > NH_3 > NO_2$

Soybean - $NO_2 > Cl_2 > NH_3$

Cotton - $NH_3 > NO_2 > Cl_2$ (about same)

Cowpea - $NO_2 > Cl_2 > NH_3$

Corn - $Cl_2 > NH_3 > NO_2$

Thus the five species show a different degree of sensitivity to the three gases.

Table 15 depicts the injury symptoms in outline form.

3. Discussion:

It is difficult in an experimental setup involving a variety of compounds and plant species, to develop a complete discussion on the effects of each chemical on each species. In addition, we have completed only a survey of the chemical effects and are in no position to draw definite or even very tentative conclusions. However, there are several things which should be discussed even though they show readily in the tabular results.

a. Germination (percent):

There are several factors which undoubtedly effect the rate

TABLE 15. Plant injury after fumigation with NH_3, NO_2 and Cl_2.

Plant	NH_3 (99 ppm)			NO_2 (81 ppm)			Cl_2 (62 ppm)		
	First Symptom	Degree of Injury*	Plant Death**	First Symptom	Degree of Injury*	Plant Death**	First Symptom	Degree of Injury	Plant Death**
Squash	(18 min)			(22 min)			(13 min)		
1 hour	interveinal chlorosis of young mature leaves	1-2	0	interveinal chlorosis of young mature leaves	1-2	0	loss of turgor, interveinal necrosis of young mature leaves	3-4	0
3 hours		4	0		3-4	0		5	6
Soybean	(38 min)			(22 min)			(13 min)		
1 hour	slight loss of turgor	1-2	0	upcupping of trifoliates	3-4	1	loss of turgor	2-3	0
3 hours		3-4	0		5	5		4	0
Cotton	(23 min)			(10 min)			(13 min)		
1 hour	appearance of pigmented areas (gossypols)	2-3	0	loss of chlorophyll in young mature leaves	2-3	0	brown necrosis of cotyledons and leaves - along margins	2-3	0
3 hours		4	0		4	0		4	0
Cowpea	(35 min)			(26 min)			(13 min)		
1 hour	interveinal chlorosis	2-3	1	primary leaves greenish-brown	2-3	0	loss of turgor and interveinal necrosis	2-3	0
3 hours		3-4	0		4	0		4	0
Corn	(35 min)			(30 min)			(25 min)		
1 hour	slight curl plus chlorosis of leaves	1-2	0	leaf curl and browning along veins	1-2	0	interveinal browning of older leaves	5	2
3 hours		5	6		4-5	3		5	6

* The degree of injury will be based on a value of 5. With 0 as no injury and 5 as death.

** Number of plants which were killed (of 6) in the three hours of fumigation after 11 days in the greenhouse (of 2 in the one hour fumigation).

and percent germination of the various seed.

1) Solution pH will effect germination where the solution actually penetrates the seed coat. In general, pH values above 9-10 and below 3-4 probably would effect seed germination. There are several examples (HCl, HNO_3, Cl_2, Br_2, UDMH, NH_3) where the solution pH either does not effect germination or the solution does not penetrate the seed coat. In several instances there appears to be a stimulation of germination with the low pH values. Only in the case of Li_2O does pH seem to be a consistent factor. This is true of KCN but there are no neutral checks on the cyanide and due to cyanides known effect on respiration we would suggest that this is a direct effect of the chemical and not of pH.

2) Effects of a specific ion on some phase of plant metabolism. It is difficult to assess the effect of individual ions. Only the Li^+ and CN^- seem to produce consistent inhibition of germination. All other ionic species produce a variety of effects.

3) Effects of specific chemical compounds on some phase of plant metabolism. Only BF_3 and LiF produce a consistent inhibition of germination in all test species. Other chemicals tend to inhibit some seed and not others. In several cases a stimulation was noted. It is impossible to catalogue all the various effects and implications noted in these studies.

4) Interactions of specific ionic components may produce some of the effects shown. As an example neither the neutralized Li_2O nor the AlF_3 cause a major decrease in germination, however, the LiF completely inhibited germination in four species and showed only 4% germination in the other species.

5) Boron trifluoride shows a possible pH interaction since the neutralized BF_3 is not nearly as inhibitory as the unneutralized.

6) The organic amines do not appear to be extremely toxic. This may be due to seed coat impermeability.

7) A comparison of the effects of the 25 test solutions on the rate of germination in the five test species would show that cowpea is the most sensitive, followed by soybean, cotton, squash and corn.

b. Growth of germinated seedlings:

Once the seed has germinated, the newly developing radicle (root) will be in direct contact with the test solution and no longer protected by the seed coat. The increased inhibition of growth in length over the percent inhibition of germination by many test chemicals attests to the protective nature of the seed

coat. In these studies it is noteworthy that all of the lithium compounds caused a virtual cessation of growth in all test species. With several exceptions this retarding of growth is also true for the fluoride containing compounds. The KCN, BF_3 and NO_2 also stop growth of germinated seeds. The boron containing compounds caused a definite growth inhibition in squash and soybean but not all of the boron compounds cause inhibition in the other three species. Hydrazine completely inhibits growth in soybean; inhibits growth in squash and cowpea; while in cotton and corn, hydrazine strongly inhibits growth at pH 6.5 but has little effect at pH 9.8. This latter effect is possibly due to the movement of more hydrazine into the plant tissues at the lower pH. UDMH, where there is a difference in effect, inhibits growth more at the higher pH than at a pH of 6.5.

All seedlings which were inhibited in their growth response developed some type of growth abnormality. A common effect was an increase in diameter of the root. Several chemicals caused death of the root tips but no special note of this effect has been included in the results.

The effect of pH on growth of the seedlings is well shown by HCl, HNO_3 and NH_3. None of these chemicals would be toxic if applied as salts at pH 5-8. Thus, the effects are most likely due to pH.

A comparison of the effects of the 25 test solutions on the growth of germinated seed of five plant species shows that squash is the most sensitive followed by soybean, cotton, corn and cowpea.

c. Soil drench studies:

Ten to 16-day old seedlings growing in a peat-perlite mix show little effect of the test compounds at 100 ppm in the week study time. The six chemicals producing injury symptoms all show an effect on the squash. Thus we might suggest that the squash plant is the most sensitive, of the plants studied, to soil applications of the test substances. It is of interest that those chemicals showing plant injury are the same group which gave pronounced inhibition of germination or seed development. Here again the lithium,boron and fluorides were toxic.

There was no pH effect in these studies due to the buffering action of the nutrient solution used.

These studies should be carried out over a longer period of time at a much lower test chemical concentration.

d. Fumigation studies:

These studies have been discussed briefly under results. The concentrations used were much too high for a thorough study. These

should be rerun at 1-10 ppm for several days or for a one-week test period.

F. Aquatic Life and Water Supplies

1. Methods and Materials:

a. Water supply:

Two sources of water were used. Source A - a small permanent pond on College property was used for the first few experiments. The pH range for source A water was 7.1 to 7.5, carbonate alkalinity was 0, bicarbonate alkalinity was 40 ppm.

Excessive silting caused by nearby construction forced abandonment of this source.

Source B, local tap water, was used for subsequent experiments. The original source of this water is from deep wells approximately 15 miles from the campus. The tap water was aerated for at least 5 days before use and tested negative for chlorine, chemically and by bio-assay, at time of use. The pH range for source B water was from 8.4 to 8.8 with the majority of the readings 8.7 to 8.8. Carbonate alkalinity was 28 ppm and bicarbonate alkalinity was 245 ppm.

The source water is indicated for each experiment.

b. All experiments were carried out in an air-conditioned room. Water temperatures varied between 70° and 75°F.

c. Goldfish:

Fish were picked up from the supplier 5 to 7 days prior to the beginning of the experiment and held in a large aquarium. The fish were fed commercial goldfish food. Feeding was stopped 48 hours prior to the start of the experiment and no feeding was done during the experiment.

For 25 fish on August 24: average length was 53.3 mm, range from 44-63 mm; average weight was 6.11 gms, range from 4.0-9.0 gms. For 37 fish on June 29, 1961: average length was 53.2 mm, range from 46-60 mm; average weight was 5.15 gms, range from 3.3-6.7 gms.

The experimental containers were 8 liter cylindrical Pyrex battery jars. Water was added to the jars, 6 liters for the control and 5 to 5.5 liters for the test solutions depending upon the volume of concentrate to be used. Concentrated solutions of the chemical to be studied were added to give a final volume of 6 liters in all 5 jars; control, 1000 ppm, 100 ppm, 10 ppm and 1 ppm. The solutions were thoroughly agitated and solutions for use in Daphnia and insect experiments were withdrawn. The total volume withdrawn from any one jar did not exceed 200 ml. Treat-

ments were not replicated.

Aeration of each solution was obtained by passing air through a standard aquarium aeration stone. One stone was used per jar and aeration was started just prior to adding the 10 goldfish. The pH was taken initially and daily for most experiments. The experiments were terminated after 72 hours.

Toxicity was evaluated by recording mortality. The fish were considered to be dead when there was no movement and no reaction to being touched with a probe.

Mortality was recorded during the first hour and at 24, 48 and 72 hours for all experiments, with occasional observations at other times.

Experiments with compounds or concentrations which might give fumes toxic to personnel were conducted in our walk-in hood.

d. Daphnia:

Daphnia cultures were maintained in 2 liter cylindrical Pyrex jars and were fed with yeast. No food was added to the cultures for 24 hours prior to the experiments and the Daphnia were not fed during the experiment. Control and test solutions (70-80 ml of each) were obtained from the solutions prepared for the goldfish experiments. Each solution was added to a 100 ml beaker to give a series of four test solutions plus the control (1000, 100, 10, 1, 0 ppm). Ten adult Daphnia were then added to each solution.

Initial and daily pH readings were taken for some but not all experiments. Toxicity was evaluated by mortality of the Daphnia. The Daphnia were considered to be dead or moribund when they had stopped swimming and ceased to move.

Mortality was recorded during the first hour and at 24, 48 and 72 hours. Mortality during the first hour was established by counting dead or moribund Daphnia. For the other mortality periods the contents of the beakers were poured into dishes and, after some of the solution was returned to the beaker, the Daphnia were counted back by drawing them one or two at a time into a plastic tube using suction from a rubber bulb and slowly expelling them back into the beaker. The remainder of the experimental solution was then returned to the beaker.

e. Odonata nymphs:

The experimental beakers were filled from the goldfish solutions and the Odonata nymphs added; the number of nymphs used varied from two to four per beaker. Observations were made at 24, 48 and 72 hours. Toxicity was evaluated by death of the nymphs. The nymphs were considered to be dead or moribund when

they did not move or respond on being touched.

f. Toxicity evaluation units:

Toxicities were evaluated by calculating median tolerance limits (TLm) at stated time intervals (24, 48 and 72 hours) by the graphical interpolation method given by Doudoroff (13). The median tolerance limit is the concentration at which 50 percent of the test organisms are able to survive for the specified period of exposure.

2. Results:

The results of the aquatic studies are presented in tabular form in Tables 16-18. TLm values for all experiments are listed in Tables 19 to 21.

3. Discussion:

It should be emphasized that the experimental work done here was of a survey nature. The results can be extrapolated only in general and with caution. The data are valid only for the organism and water types used. The influence of water chemistry is demonstrated by differences of a factor of 10, in some cases, for the lethal concentration in the two water types used. Goldfish are relatively resistant to toxic chemicals in comparison to other fresh water species, particularly those of sport value. For example, in a preliminary experiment with bluegill (Lepomis macrochirus, Rafinesque) the TLm for N_2H_4 was 3.2 ppm compared with 10 ppm for goldfish. However, due to the 10-fold variation in the four concentrations used, the TLm values are only approximate.

The experiments have yielded good data on the general order or magnitude of the toxicity of the chemicals tested and of their relative toxicities, and provide a good basis for the design of more precise experiments.

a. The effect of pH:

In general, where pH values were obtained they tended to change in the direction of neutrality. Salts showed slight changes which were probably due to the effect of the fish. The pH of gases and volatile liquids in solution changed the most - possibly due to loss of gas by aeration.

The changes in the small unaerated beakers were less than in the aerated jars. The differences between measurements on the same chemical after a given time period were usually less than 0.5 pH units.

The buffering action of the alkaline "B" water gave a slower increase or decrease in pH as concentration increased, and resulted in less extreme pH values at the highest concentration.

TABLE 16. The toxicity of the test chemicals to goldfish. Ten goldfish were used at each concentration.

Chemical	Water source	Concentration/ppm	pH at start	Number of organisms alive and pH at indicated hrs. after initiation of exp.						
				1	24		48		72	
				No. alive	pH	No. alive	pH	No. alive	pH	No. alive
Li_2O	A	Control	7.4	10	7.9	10	-	10	-	10
	B	Control	8.75	10	-	10	-	10	8.5	10
	A	1	7.75	10	7.45	10	-	10	-	10
	B	1	8.9	10	-	9	-	9	8.4	9
	A	10	10.5	10	7.25	10	-	10	-	10
	B	10	9.5	10	-	10	-	10	8.5	10
	A	100	11.7	0						
	B	100	11.4	0						
	A	1000	12.4	0						
	B	1000	12.2	0						
LiF	A	Control	7.1	10	-	10	-	10	-	10
		1	7.1	10	-	10	-	10	-	10
		10	7.2	10	-	10	-	10	-	10
		100	7.3	10	-	10	-	8	-	2
		1000	7.5	10	-	5	-	0	-	
AlF_3	A	Control	7.2	10	7.7	10	-	10	7.3	10
		1	7.1	10	7.5	10	-	10	7.2	10
		10	6.5	10	7.1	10	-	10	7.0	10
		100	6.3	10	6.7	7	-	5	-	0
		1000	6.1	10	-	0				
Al_2O_3	A	Control	7.2	10	7.3	10	-	10	7.4	10
		1	7.2	10	7.3	10	-	10	7.3	10
		10	7.2	10	7.4	10	-	10	7.4	10
		100	7.2	10	7.4	10	-	10	7.5	10
		1000	7.2	10	7.4	10	-	10	7.4	10
B_2O_3	A	Control	7.5	10	-	10	-	10	-	10
		1	7.4	10	-	10	-	10	-	10
		10	7.4	10	-	10	-	10	-	10
		100	7.2	10	-	10	-	10	-	10
		1000	6.8	10	-	10	-	6	-	6
N_2H_4	A	Control	7.5	10	-	10	-	10	-	10
	B	Control	8.8	10	-	10	-	10	8.5	10
	A	1	7.4	10	-	10	-	10	-	10
	B	1	8.8	10	-	9	-	9	8.3	9
	A	10	8.0	10	-	5	-	0		
	B	10	8.8	7	-	0				
	A	100	8.8	5	-	0				
	B	100	9	5	-	0				
	A	1000	9.6	0						
	B	1000	9.3	0						

(continued)

Table 16. (continued)

Chemical	Water source	Concentration/ppm	pH at start	Number of organisms alive and pH at indicated hrs. after initiation of exp.					
				1	24		48		72	
				No. alive	pH	No. alive	pH	No. alive	pH	No. alive
NH_4OH	A	Control	7.5	10	7.5	10	7.2	10	7.5	10
	B	Control	8.7	10	8.8	10	-	10	8.4	10
	A	1	8.1	10	7.4	10	7.4	10	7.4	10
	B	1	8.7	10	8.8	10	-	10	8.4	10
	A	10	9.2	10	7.8	10	7.6	10	7.6	10
	B	10	9.0	10	8.7	9	-	9	8.4	9
	A	100	10.0	0						
	B	100	9.6	1	-	0				
	A	1000	10.7	0						
	B	1000	10.2	0						
HCl	A	Control	7.4	10	7.5	10	7.4	10	7.5	10
	B	Control	8.7	10	8.8	10	-	10	8.4	10
	A	1	7.0	10	7.0	10	7.2	10	7.4	10
	B	1	8.7	10	8.7	10	-	10	8.3	10
	A	10	4.6	10	6.8	10	7.3	10	7.4	10
	B	10	8.2	10	8.5	10	-	10	8.2	10
	A	100	2.8	0						
	B	100	6.8	10	8.2	10	-	10	7.9	10
	A	1000	2.0	0						
	B	1000	2.4	0						
HNO_3	B	Control	8.4	10	7.8	10	7.6	10	7.6	10
		1	8.4	10	7.8	10	7.6	10	7.7	10
		10	8.0	10	7.6	10	7.6	10	7.8	10
		100	6.7	10	7.3	10	7.3	10	7.5	10
		1000	2.1	0						
$NaNO_2$	B	Control	8.4	10	7.8	10	7.6	10	7.6	10
		1	8.4	10	7.7	10	7.5	10	7.7	10
		10	8.4	10	7.9	10	7.7	10	7.9	10
		100	8.4	10	7.8	10	7.6	10	7.5	10
		1000	8.2	10	8.0	1		0		
UDMH	B	Control	8.5	10	8.2	10	7.7	10	7.7	10
		1	8.5	10	8.0	10	7.8	10	7.7	10
		10	8.5	10	8.0	10	7.7	10	7.6	10
		100	8.7	10	8.1	4	-	0		
		1000	9.4	10	-	0				
NH_4ClO_4	B	Control	8.6	10	8.2	10	7.7	10	7.7	10
		1	8.6	10	8.0	10	7.9	10	7.8	10
		10	8.5	10	8.1	10	8.0	10	7.9	10
		100	8.3	10	8.2	9	8.0	9	7.8	9
		1000	7.7	8	-	0				

(continued)

Table 16. (continued)

Chemical	Water source	Concentration/ppm	pH at start	Number of organisms alive and pH at indicated hrs. after initiation of exp.					
				1	24		48		72	
				No. alive	pH	No. alive	pH	No. alive	pH	No. alive
NO_2	B	Control	8.7	10	9.2	10	9.0	10	8.9	10
		1	8.7	10	9.2	10	9.0	10	9.2	10
		10	8.4	10	9.1	10	8.8	10	9.0	10
		100	6.8	10	8.9	10	8.7	10	8.9	10
		1000	2.4	0						
$K_2B_4O_7$	B	Control	8.5	10	9.2	10	9.0	10	8.7	10
		1	8.5	10	9.6	10	9.2	10	9.1	10
		10	8.7	10	9.2	10	9.0	10	9.1	10
		100	8.8	10	9.4	10	9.0	10	9.1	10
		1000	8.9	0						
Cl_2	B	Control	8.8	10	8.7	10	8.7	10	8.7	10
		1	8.8	10	8.7	9	8.6	9	8.6	9
		10	8.6	10	-	0				
		100	7.4	0						
		1000	3.4	0						
Br_2	B	Control	8.8	10	8.7	10	8.7	10	8.7	10
		1	8.7	10	8.6	9	8.2	9	8.4	9
		10	8.6	7	8.5	6	8.1	5	8.2	4
		100	8.0	0						
		1000	6.8	0						
BF_3	B	Control	8.6	10	8.6	10	8.2	10	8.0	10
		1	8.7	10	8.7	10	8.3	10	8.2	10
		10	8.3	10	8.2	10	7.7	10	7.6	10
		100	6.3	10	7.0	10	6.8	10	7.8	10
		1000	2.5	0						
HF	B	Control	8.6	10	8.6	10	8.2	10	8.0	10
		1	8.5	10	8.5	10	8.3	10	8.2	10
		10	7.5	10	7.2	10	7.2	10	7.1	10
		100	4.2	3	-	0				
		1000	2.9	0						

TABLE 17. The toxicity of the test chemicals to Daphnia pulex. Ten Daphnia were used at each concentration.

Chemical	Water source	Concentration/ppm	pH at start	Number of organisms alive and pH at indicated hrs. after initiation of exp.						
				1		24		48		72	
				No. alive	pH	No. alive	pH	No. alive	pH	No. alive
Li_2O	B	Control	8.8	10	-	5	-	5	8.5	4
		1	8.9	10	-	8	-	8	8.4	7
		10	9.5	10	-	0				
		100	11.4	0						
		1000	12.2	0						
LiF	B	Control	8.8	10	-	10	-	8	8.4	8
		1	8.8	10	-	0				
		10	8.8	10	-	6	-	4	8.4	2
		100	8.8	10		0				
		1000	8.8	5		0				
AlF_3	A	Control	7.2	10	-	10	-	0		
		1	7.1	10	7.3	10	-	10	-	10
		10	6.5	10	7.0	10	-	5	-	0
		100	6.3	10	6.4	2	-	0		
		1000	6.1	10		0				
Al_2O_3	A	Control	7.2	10	-	10	-	0		
		1	7.2	10	-	10	-	10	-	10
		10	7.2	10	-	10	-	10	-	10
		100	7.2	10	-	10	-	10	-	10
		1000	7.2	10	-	10	-	10	-	10
B_2O_3	A	Control	7.5	10	-	10	-	8	-	8
		1	7.4	10	-	10	-	9	-	9
		10	7.4	10	-	9	-	9	-	9
		100	7.2	10	-	10	-	9	-	8
		1000	6.8	10	-	6	-	0		
N_2H_4	A	Control	7.5	10	-	10	-	10	-	10
	B	Control	8.8	10	-	9	-	7	8.3	7
	A	1	7.2	10	-	9	-	8	-	7
	B	1	8.8	10	-	6	-	5	8.3	1
	A	10	8.0	10	-	0				
	B	10	8.8	10		0				
	A	100	8.8	0						
	B	100	9.0	0						
	A	1000	9.6	0						
	B	1000	9.3	0						
UDMH	B	Control	8.5	10	7.8	10	7.3	10	7.2	10
		1	8.5	10	7.9	10	7.4	10	7.3	9
		10	8.5	10	8.1	10	7.7	8	7.5	4
		100	8.7	10	8.2	0				
		1000	9.4	9	8.7	0				

(continued)

Table 17 (continued)

Chemical	Water source	Concentration/ppm	pH at start	Number of organisms alive and pH at indicated hrs. after initiation of exp.					
				1	24		48		72	
				No. alive	pH	No. alive	pH	No. alive	pH	No. alive
NH_4OH	A	Control	7.5	10	-	10	7.3	10	7.3	10
	B	Control	8.7	10	9.0	10	-	6	8.3	0
	A	1	8.1	10	-	10	7.4	10	7.3	10
	B	1	8.7	10	8.9	10	-	10	8.6	0
	A	10	9.2	10	-	3	7.5	2	7.3	2
	B	10	9.0	10	9.0	10	-	9	8.7	0
	A	100	10.0	0						
	B	100	9.6	0						
	A	1000	10.7	0						
	B	1000	10.2	0						
HCl	A	Control	7.4	10	-	7	7.3	7	7.4	7
	B	Control	8.7	10	8.8	10	-	10	8.4	7
	A	1	7.0	10	-	8	7.2	8	7.4	8
	B	1	8.7	10	8.8	10	-	9	8.4	0
	A	10	4.6	10	-	9	7.1	9	7.4	2
	B	10	8.2	10	8.6	9	-	7	8.4	5
	A	100	2.8	0						
	B	100	6.8	10	8.2	10	-	8	8.2	6
	A	1000	2.0	0						
	B	1000	2.4	0						
HNO_3	B	Control	8.4	10	7.6	7	7.1	7	7.6	5
		1	8.4	10	7.7	9	7.2	6	7.7	2
		10	8.0	10	7.7	8	7.2	7	7.4	7
		100	6.7	10	7.2	0				
		1000	2.1	0						
$NaNO_2$	B	Control	8.4	10	7.8	9	7.3	8	7.2	8
		1	8.4	10	7.8	9	7.4	6	7.4	6
		10	8.4	10	7.8	8	7.4	7	7.5	6
		100	8.4	10	7.8	8	7.5	0		
		1000	8.2	10	7.6	0				
NH_4ClO_4	B	Control	8.5	10	8.3	9	7.7	8	7.5	8
		1	8.5	10	8.3	9	7.7	7	7.5	4
		10	8.5	10	8.2	10	7.6	9	7.6	8
		100	8.7	10	8.1	10	7.6	9	7.6	7
		1000	9.4	10	7.7	0				
NO_2	B	Control	8.7	10	9.0	10	8.6	7	8.8	7
		1	8.7	10	9.1	9	8.7	9	8.9	8
		10	8.4	10	9.2	10	8.8	7	9.0	6
		100	6.8	10	8.6	10	8.4	10	8.7	7

(continued)

Table 17. (continued)

Chemical	Water source	Concentration/ppm	pH at start	Number of organisms alive and pH at indicated hrs. after initiation of exp.					
				1	24		48		72	
				No. alive	pH	No. alive	pH	No. alive	pH	No. alive
NO_2	B	1000	2.4	0						
$K_2B_4O_7$	B	Control	8.5	10	9.2	10	9.0	8	9.0	8
		1	8.5	10	9.2	8	9.0	8	9.0	7
		10	8.7	10	9.4	9	9.1	9	9.2	7
		100	8.8	10	9.6	6	9.3	5	9.4	3
		1000	8.9	5	-	0				
Cl_2	B	Control	8.8	10	8.7	9	8.6	8	8.6	7
		1	8.8	5	-	0				
		10	8.6	0						
		100	7.4	0						
		1000	3.4	0						
Br_2	B	Control	8.8	10	8.9	10	8.7	10	8.7	9
		1	8.7	10	8.8	10	8.8	9	8.8	9
		10	8.6	5	-	0				
		100	8.0	0						
		1000	6.8	0						
BF_3	B	Control	8.6	10	7.5	6	7.0	0		
		1	8.7	10	7.4	10	6.9	10	6.9	9
		10	8.3	10	7.4	9	6.9	9	6.9	9
		100	6.3	10	6.9	6	6.4	6	6.3	6
		1000	2.5	0						
HF	B	Control	8.6	10	7.5	6	7.0	6	7.0	6
		1	8.5	10	7.4	9	6.9	9	6.9	7
		10	7.5	10	7.4	10	6.9	6	6.8	4
		100	4.2	2	-	0				
		1000	2.9	0						

TABLE 18. Toxicity of some test chemicals to Odonata nymphs.

Chemical	Water source	Concentration/ppm	Initial no. of Odonata	Initial pH	Number of organisms alive at indicated hours after initiation of experiment		
					24	48	72
Li_2O	B	Control	3	8.8	2	2	1
		1	3	8.9	3	3	3
		10	2	9.5	2	1	1
		100	3	11.4	2	2	0
		1000	3	12.2	0		
NH_4OH	B	Control	1	8.7	1	1	1
		1	1	8.7	1	1	1
		10	2	9.0	1	0	
		100	2	9.6	1	0	
		1000	2	10.2	0		
Br_2	B	Control	2	8.8	2	2	2
		1	2	8.7	2	2	2
		10	2	8.6	2	1	1
		100	2	6.8	0		
		1000	2	6.8	0		
$K_2B_4O_7$	B	Control	1	8.5	0		
		1	2	8.5	2	2	2
		10	1	8.7	1	1	1
		100	2	8.8	2	2	2
		1000	2	8.9	1	1	0
HNO_3	B	Control	3	8.4	3	2	2
		1	3	8.4	3	1	1
		10	3	8.0	3	3	3
		100	3	6.7	3	3	3
		1000	4	2.1	2	0	
$NaNO_2$	B	Control	3	8.4	3	3	3
		1	2	8.4	2	1	1
		10	3	8.4	3	3	3
		100	3	8.4	3	1	1
		1000	2	8.2	2	0	
B_2O_3	A	Control	3	7.5	3	3	2
		1	2	7.4	2	2	2
		10	2	7.2	2	2	2
		100	2	6.8	3	3	2
N_2H_4	A	Control	3	7.5	3	3	3
		1	2	7.4	2	2	2
		10	2	8.0	2	2	2
		100	2	8.8	2	2	1
		1000	3	9.6	0		

TABLE 19. TLm values in parts per million for goldfish. Asterisk indicates no mortality at highest test concentration, 1000 ppm.

Chemical	Water source	Length of experimental period in hours		
		24	48	72
Li_2O	A	32	32	32
Li_2O	B	32	32	32
LiF	A	1000	230	42
AlF_3	A	190	100	32
AL_2O_3	A	*	*	*
B_2O_3	A	*	1800	1800
N_2H_4	A	10	3.2	3.2
N_2H_4	B	3.2	2.8	2.8
NH_4OH	A	32	32	32
NH_4OH	B	28	28	28
HCl	A	32	32	32
HCl	B	320	320	320
HNO_3	B	320	320	320
$NaNO_2$	B	360	320	320
UDMH	B	69	32	32
NH_4ClO_4	B	280	280	280
NO_2	B	320	320	320
$K_2B_4O_7$	B	320	320	320
Cl_2	B	2.8	2.8	2.8
Br_2	B	15	10	7.9
BF_3	B	320	320	320
HF	B	32	32	32

TABLE 20. TLm values in parts per million for Daphnia pulex (de Geer). Asterisk indicates no mortality at highest test concentration, 1000 ppm.

Chemical	Water source	Length of experimental period in hours		
		24	48	72
AlF_3	A	42	10	3.2
AL_2O_3	A	*	*	*
B_2O_3	A	4300	280	240
N_2H_4	A	208	2.4	1.9
N_2H_4	B	1.7	1.0	.2
UDMH	B	32	24	6.1
NH_4OH	A	5.1	4.2	4.2
NH_4OH	B	32	26	-
HCl	A	28	24	4.3
HCl	B	320	240	150
HNO_3	A	24	19	19
$NaNO_2$	B	240	19	15
NH_4ClO_4	B	320	280	190
NO_2	B	320	320	190
$K_2B_4O_7$	B	150	100	32
Cl_2	B	.27	-	-
Br_2	B	3.2	2.8	2.8
BF_3	B	150	150	150
HF	B	32	15	4.7
LiF	B	15	-	-
Li_2O	B	2.4	2.4	1.9

TABLE 21. TLm values in parts per million for Odonata nymphs, family Libellulidae. Asterisk indicates no mortality at highest test concentration, 1000 ppm.

Chemical	Water source	Length of experimental period in hours		
		24	48	72
Li_2O	B	170	170	5.5
NH_4OH	B	100	3.2	3.2
Br_2	B	100	10	10
$K_2B_4O_7$	B	1000	1000	320
HNO_3	B	1000	320	320
$NaNO_2$	B	*	56	56
B_2O_3	A	*	*	*
N_2H_4	A	320	320	100

b. Chemical effects on goldfish:

Over the 72 hours of the tests, only three chemicals gave mortality at 1 ppm; N_2H_4 (B water), Cl_2 and Br_2. Only two additional chemicals gave mortality at 10 ppm; N_2H_4 (A water) and NH_4OH (B water).

Mortality was 100 percent for all chemicals tested at 1000 ppm except Al_2O_3 and B_2O_3; Al_2O_3 gave no mortality, B_2O_3 partial.

For the other 15 experiments the toxicity threshold lies between 10 and 1000 ppm.

For 12 chemicals mortality ceased after 24 hours. Three chemicals (N_2H_4, UDMH, $NaNO_2$) had some additional mortality at 48 hours but none at 72 hours; only Br_2, AlF_3 and LiF had continuing mortality during the 72 hours. In several cases this was probably due to loss of chemical from the test treatment.

c. Chemical effects on Daphnia:

Although the data up to 72 hours are presented in Table 17, the reactions of the controls indicate that 72 hours is probably too long under the experimental conditions used here. Thus, the 72 hour data will not be discussed.

No mortality occurred at any concentration of Al_2O_3. All but four of the remaining chemicals, AlF_3, UDMH, NH_4OH (A & B) and BF_3, gave some mortality at 1 ppm.

With the exception of Al_2O_3, mortality was total at 1000 ppm for all chemicals and mortality was total or partial at 100 ppm for all except NO_2.

As compared to goldfish, the toxicity threshold is much lower and the TLm values are lower by 1/2 to 1/5. The fact that the goldfish experiments were aerated and the Daphnia not aerated must be considered, in addition to inherent differences between the organisms.

d. Chemical effects on dragonfly nymphs:

Of the 8 chemicals tested, B_2O_3 gave partial mortality and the other 7 total mortality at 1000 ppm.

The TLm values in Table 21 must be considered only approximations becuase of the small number of organisms per experiment.

In general, the Dragonfly nymphs appear to be somewhat more resistant than the goldfish to the chemicals tested, and in the case of N_2H_4 markedly so. An aquatic mite, order Hydracarina, was introduced with the dragonfly nymphs in one experiment with N_2H_4 at 1000 ppm. The mite lived 24 hours longer than the Dragonfly nymphs, evidence perhaps for an even more resistant group.

e. Additional comments:

Consideration of the results presented here and of the probably general ways in which the chemicals could be introduced into the natural environment point out some important areas for continued investigation.

1) Stream pollution:

When the chemical enters in one application, the organisms will be exposed to maximum concentrations for a relatively short period of time as the water mass moves downstream. The concentration at any one point behind this water mass will depend upon volume of flow, initial concentration, and the extent of backwaters which may be slow to flush. Concentration in the initial water mass may be decreased by dilution, by the loss of volatile components or by neutralization reactions.

Effects of contamination of this sort may be reduced by impounding the contaminated water until toxicity is reduced to a safe level or by increasing stream flow by release of impounded uncontaminated water with resulting dilution and increase in the rate of movement of the contaminated water mass.

The importance of information about short term exposures to lethal concentrations is evident upon consideration of the extent of mortality after only one hour. Further evidence is provided by two experiments. In the first experiment, 6 goldfish were dipped for 10 seconds into 1000 ppm Li_2O and then placed in control water. All were dead in one hour. In the second experiment, 6 goldfish were dipped for 10 seconds into 1000 ppm N_2H_4 and then placed in control water. All were dead in 4 hours.

A second type of stream contamination would result from continuous leaching of the chemical from contaminated soil or other areas by runoff water, or by leakage from tanks or containers. This would result in a relatively low but constant concentration of the chemical. The data presented here indicate that for many chemicals aeration or neutralization caused a decrease of the toxic component during the experiment. TLm levels established in this way would be too high for conditions of constant concentration level. This aspect of toxicity should be investigated using constant flow conditions over relatively long exposure times.

2) Lake and pond pollution:

In most cases removal of the contaminated water by flushing would not be possible and if possible might not be desirable because of downstream effects. The same considerations would apply to releasing the contaminated water.

An understanding of low level continuous exposure is even more necessary here than for stream conditions.

Consideration should also be given to the effects of aeration and neutralization.

If adequate information is to be obtained for an understanding of the possible environmental affects of the chemicals studied, and to enable the presentation of handling procedures and procedures in the event of contamination, the general TLm values and toxicity data presented in this report are only a first step.

G. Recommendations for Future Study

1. Additional Studies on Soil Microflora:

a. The chemicals should be studied in groups of 3-4.

b. Following procedures already established; samples should be obtained from the treated and the untreated soil. Easily leached chemicals would be effective on the lower soil studies.

c. Test chemicals should be incubated with the soil for longer periods of time and two sampling dates used.

d. The chemicals should be studied at several concentrations to determine the lower limit of their effect on the microflora. These studies should be incorporated with time studies to obtain some concept of time concentration interrelationships.

e. Effects of the listed toxicants on populations of specific soil organisms should be studied.

f. Long term studies of incubated soil, under various environmental conditions, including differing degrees of soil fertility, should be undertaken.

g. Detailed studies, listed above, should be undertaken only with these chemicals showing a definite effect on soil microflora at a given concentration.

2. Additional Studies on Soil, Soil Structure and Runoff Water:

a. A detailed study of the effect of the mineralogical composition of clays upon the adsorption of the test compounds needs to be undertaken. This is essential before a sound interpretation of the data can be rendered. This data would be of importance in understanding the plant response and soil microflora responses as well as for the soil studies.

b. Runoff studies should be continued. The following variations are recommended: minimum slope of 2%; a mulched surface and a hard, natural surface; and, an 18 inch wide by 50 foot long plot. Both soil and water samples will be taken at 15, 30 and 45 foot intervals to determine the transfer and/or adsorption of the test compound down the slope. Water will be applied at the head of the plot by adjustable spray nozzles to simulate rainfall conditions. The entire plot will be wet with 1 inch of water, before application of treatment, to assure maximum runoff conditions. These studies should be directed toward determining the fate of the individual chemical after it is applied to the soil.

c. Water leaching studies should be carried further, especially with those chemicals which leached in this year's study. Soil will be sampled by 6 inch depth increments and the following analyses made: pH of sample and concentration of the chemical. Both concentration and pH of effluent will be determined. These studies should be continued in the laboratory and possibly extended to field conditions - a field study should cover at least a 2-year period. Soils to be used should consist of deep, coarse-textured, freely permeable sands or sandy loams because these are the types in which the greatest amounts of water will percolate and eventually reach the lower pumping strata.

d. The soil aggregate studies should be continued using several stronger concentration levels. The incubation period should be extended and periodic counts of soil bacteria and fungi should be made.

3. Additional Studies on Plant Growth and Development:

a. Experimental results obtained in the first year of study should fairly well delimit those chemicals which require additional study. These chemicals should be submitted to further investigative procedures.

b. Long term experiments: plants would be grown from seed to maturity under continuous exposure to low concentrations of toxic compounds. This would be especially important for soil application studies.

c. Periodic exposure of plants to various concentrations of toxicant over a long period of time - preferably the life of the plant.

d. Environmental studies: by use of specially constructed growth chambers, the effects of environmental variations on the toxicity symptoms of specific chemicals can be determined. Where chemicals will be used throughout the year, it would be important to know the effects of different temperatures and humidities on plant growth and development.

e. Additional plant species should be investigated to establish indicator species which could be used as test plants to predict harmful concentrations of toxicants.

f. Interaction of the different toxicants should be investigated. The air pollution aspects of this type of study could be most productive. Studies to date have shown that the photochemical oxidation of hydrocarbon mixtures often produces substances more toxic than the original organic toxicant.

g. A systematic analysis of plant tissues for chemical residues should be undertaken to determine possible indirect effects of the test chemicals on man and domestic and wild animals. Plant tissues often accumulate relatively high concentrations of a chemical compound. It is possible that such residues would exist in plant parts utilized as a food source. Such concentrations could conceivably accumulate over a growing season even if air or soil concentrations were too low to cause noticeable or measurable plant injury.

h. The use of isotopes would aid the residue study, proposed above, and would enable the investigators to study, in greater detail, the biochemical effects of the propellants on the plants under study. The possibility of increasing the basic concepts of plant biochemistry further warrants the extension of this project to include the isotope studies. It is impossible to predict the practical applications of basic studies but here they definitely seem warranted.

4. Additional Studies on Aquatic Life and Water Supplies:

a. The effect of water quality, specifically alkalinity, on the

toxicity of the compounds tested needs to be studied. Our present water sources (tap or pond water) will be used with various distilled water dilutions. Each test compound will be studied with the different dilutions at a concentration known to be toxic. This would give invaluable data on the influence of water quality on toxicity.

b. Stream contamination may occur as a sludge, or water mass, which will not remain in any one location for over a few hours. Under periods of short exposure organisms may be able to survive concentrations which would be lethal over longer periods. This problem would be attacked by immersing the test organisms in solutions known to be toxic, then removing the organisms from the test solution for observations of subsequent mortality. The times of immersion in the test solutions and the concentrations of the test solutions would be varied.

c. Organisms were tested in static systems this year, the fish under constant aeration. Thus, the concentration of many test chemicals decreased with time. Under natural conditions a relatively constant rate of contamination may be caused by runoff or soil leaching. This would expose the organisms to a more uniform concentration of toxicant. The toxic levels under these conditions may be lower than those observed for a closed system. A constant flow system will be constructed which will enable us to maintain a given toxic concentration in the test solution. This system will be used in a critical study of the toxicant concentration required to induce injury in the test organisms.

d. Further studies should be run on water quality. These could involve the addition of known amounts of chemicals occurring naturally in pond or stream waters. These artificial "pond" waters could be tested with the toxicants and survival of aquatic organisms determined.

e. Many of the recommendations outlined under the plant studies would be worthwhile for the aquatic studies, of special interest would be d through h.

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APPENDIX

TABLE A-1. Tabular information from the literature review.

Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-f	.001	Several days	Plants-vegetation	2-4	2	
	L-f	.02-.05		Gladiolus, prune, apricot, peach	2-3	29	
	L-f	.005 or less	7-9 days	Pine (young needles, milo maize sorghum), corn, sweet potato	1	29	
	L-f	.005-.01	7-9 days	Many fruits	1	29	
	L-f	Greater than 0.01	7-9 days	Alfalfa, tomato, rose	1	29	
	L-f	1	1 hr or more	Plants	3	29	Interveinal and marginal acute markings

* F - field
L - laboratory
f - fumigation
w - water
n - nutrient solution
s - soil

** As ppm of compounds listed unless otherwise stated.

*** Degree of Injury
4 lethal
3 severe
2 moderate
1 slight
0 no injury

(continued)

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-f	1	Very long	Squash	1	29	Only marginal chlorosis
	L-f	.001-.05	Several hrs	Sensitive plants	2	29	Typical field symptoms
	F-w	100-500		Beans	1	17	Inhibited sprouting
	F-w	1000		Large plants	2	39	Stunted growth
	L-n	25	10-13 days	Peach	2	39	Patterns of marginal leaf injury were identical
	L-n	25	10-13 days	Buckwheat	2	39	Patterns of marginal leaf injury were identical
	L-n	50	27 days	Tomato	2	39	Patterns of marginal leaf injury were identical
	L-n	25	48 days	Tomato	2	39	Patterns of marginal leaf injury were identical
	L-n	10	10 days	Gladiolus and others	1	29	
	L-n	40	4-5 days	Gladiolus and others	1	29	
	L-n	100	1 day	Gladiolus and others	3	29	
	L-w	100		Tomato	1	43	Sprayed on leaves

(continued)

(continued)

Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-w	500		Tomato	2	43	Sprayed on leaves
	L-w	5000		Tomato	3	43	Sprayed on leaves
	L-f	2		Plants	1	43	Threshold of marking
	L-f	0.05	4-8 hrs	Gladiolus	2	44	Pronounced markings
	L-f	0.05	4-8 hrs	Maize, milo (sorghum, sp.); Prunus sp.; Italian prune, Prunus sp.	3	44	
	L-f	0.05	4-8 hrs	Buckwheat, (Fagopyrum esculentum) Moench, corn, sweet potato, apple	2	44	
	L-f	0.05	4-8 hrs	Rose	1	44	
	L-f	0.05	4-8 hrs	Cotton, tomato, alfalfa	0	44	
	L-f	0.07	4 hrs	Prune	1	39	
	L-f	0.085	6-7 hrs	Buckwheat, peach, sweet potato	1	39	
	L-f	0.1	2.2 hrs	Prune, buckwheat, sweet potato, peach, gladiolus, crabgrass, corn, bean, pine	2	39	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-f	0.67	2.2 hrs	Tomato	1	39	
	L-f	1.48	2.2 hrs	Prune, buckwheat, sweet potato, peach, gladiolus, crabgrass, corn, bean, pine, tomato	3	39	
	L-f	0.01-0.10		Sweet potato, white pine, peach, gladiolus	2	39	
	L-f	0.30-0.40		Tomato, catbriar, smartweed, crabgrass, sorrel, tobacco, begonia, geranium	2	39	
	L-f	0.40-0.50		Spinach, pepper, corn	2	39	
	L-f	1		Aster, poinsettia, ragweed, plantain, zinnia, marigold, petunia	0	39	
	L-f	0.001-0.002	Few days	Gladiolus, sensitive varieties	2-3	39	Constant flow fumigation, 1.5 changes of air per minute

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-f	Less than 0.001	Few days	Gladiolus, sensitive varieties	1-2	39	Constant flow fumigation, 1.5 changes of air per minute
	L-f	0.0001	5 weeks	Picardy var. gladiolus	1	39	Constant flow fumigation, 1.5 changes of air per minute
	L-f	0.01	22 hrs	Picardy var. gladiolus	3	39	Constant flow fumigation, 1.5 changes of air per minute
	F-f	0-0.064		Ponderosa pine	3-4	39	
	L-f	0.01-0.1 (F_2)	3 hrs	Sweet potato, white pine, peach, gladiolus	3	9	
	L-f	0.1(F_2)	3 hrs	Tomato	0	9	
	L-f	0.31(F_2)	3 hrs	Tomato	2-3	9	
	L-f	0.1-0.3 (F_2)	3 hrs	Catbriar, smartweed, wild sorrel	2-3	9	
	L-f	0.4(F_2)	3 hrs	Garden sorrel, tobacco, begonia sp., geranium sp.	2-3	9	
	L-f	0.1(F_2)	3 hrs	Garden sorrel, tobacco, begonia sp., geranium sp.	0	9	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-f	0.4-0.5 (F_2)	3 hrs	Spinach, pepper, corn	2-3	9	
	L-f	1.0(F_2)	3 hrs	Bean, aster sp., poinsettia, ragweed, broadleafed plantain, zinnia sp., marigold sp., petunia sp.	0	9	
	L-f	0.05-0.1 (F_2)	Few hrs	Elberta peach	3	18	On leaves
	L-f	Greater than 0.1 (F_2)	Several hrs	Elberta peach	3	18	Twig or fruit damage
	L-f	0.12	6 hrs	Wild black cherry shrubs	3	18	4-5% of the leaves
	L-f	0.33	4.1 hrs	Apple	0-1	18	Leaves approaching maturity
	L-f	0.15-0.28	5.6 hrs	Norway spruce	1-2	18	
	L-f	Less than 1		Buckwheat	2	18	
	L-f	3-10		Buckwheat	4	18	
	L-f	Av. 0.27 Max. 0.98	10.1 hrs	Buckwheat	3-4	18	8-10% of leaves

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Fluoride	L-f	Av. 0.19	7.6 hrs	Buckwheat	3-4	18	5% of leaves
	L-f	2-10		Tomato	2-3	18	
	L-f	Less than 2		Tomato	1-2	18	
	L-f	Av. 0.67	4.33 hrs	Tomato	0	18	Did not kill blossom or cause fruit drop
	L-f	0.23	13.6 hrs	Tomato	1	18	
	L-f	Av. 0.35 Max. 0.56	10 2/3 hrs	Corn	2-3	18	
	L-f	Av. 0.35 Max. 0.56	3.5 hrs	Buckwheat	3	18	
	L-f	Av. 0.35 Max. 0.56	3.5 hrs	Peach	2	18	
	L-f	Av. 0.35 Max. 0.56	3.5 hrs	Apple	1	18	
Sodium Fluoride	L-w	17.1	1 hr	Minnows	0	17	
	L-w	100	More than 4 days	Goldfish	0	17	
	L-w	1000	60-102 days	Goldfish	4	17	Hard water

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Sodium Fluoride	L-w	504		(Daphnia magna)	4	17	
Hydrogen Fluoride	L-w	40		Fish	2-3	17	
	L-w	60		Fish	4	17	
	L-w	358(F_2)		Trout	2-3	26	Soft H_2O
Chlorine	L-w	0.15-0.2	12-16 days	Carp	4(25%)	17	
	L-w	0.2-0.3		Fish	1	17	
	L-w	0.25	5 hrs	Fingerlings	4	17	
	L-w	0.3	2 hrs	Trout	4	17	
	L-w	0.3-10.0		Fish	4	17	
	L-w	0.8	47 min	Small trout	4	17	
	L-w	1.0	1 hr	Trout	4	17	
	L-w	1.0	4 days	Many types	4	17	
	L-w	1.0		Goldfish	4	17	
	L-w	1.0		Trout and goldfish	4	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Chlorine	L-w	2.0-4.0		Fish	4(30%)	17	
	L-w	0.1	2.5 hrs	Small trout	0	17	
	L-w	0.1		Fish	0	17	
	L-w	0.25	42 hrs	Goldfish	0	17	
	L-w	0.3	2 hrs	Minnows	0	17	
	L-w	0.5		Trout and goldfish	0	17	
	L-w	1.0		Minnows	0	17	
	L-w	1.0		Goldfish	1	17	
	L-w	1.0	100 hrs	Eels	0	17	
	L-w	1.0		Carp	0	17	
	L-w	1.0	4 hrs	Fish	2	17	Maximum concentration survived
	L-w	5.0		Goldfish	0	17	
	L-w	10	8 hr day	Mussels, anemones	3-4	17	In sea H_2O
	L-w	10	4 hr day	Barnacles	3-4	17	In sea H_2O
	L-w	10	1 hr	Tunicates and bryozoa	4	17	In sea H_2O

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Chlorine	L-w	2.5	5-8 days	Mussels, anemones, barnacles, tunicates, bryozoa	4	17	In sea H_2O
	L-w	1	15 days	Barnacles and anemones	3-4	17	In sea H_2O
	L-w	0.01-0.05		Most oysters	1-2	17	Pumping is reduced
	L-w	Greater than 1.0		Oysters	3	17	Effective pumping cannot be maintained
	L-w	2000		Perch	0	26	
	L-w	0.05-0.2		Fish	4	26	
	L-w	0.3		Trout	4	26	
	L-w	0.03	6 hrs	(Salmo iridius, Gibbons) rainbow trout	0-1	26	Overturned
	L-w	2		(Carassium auratus, L.) goldfish	4	26	18-23°C.
	L-w	1.5, 2.0, 3.0		Goldfish	4	8	H_2O changed daily
	L-w	1.0, 1.5		Goldfish	4	8	H_2O constantly renewed

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Chlorine	L-w	1		Sensitive fish	4	13	
	L-w	Less than 0.2		Rainbow trout	0	37	Tap H_2O
	L-w	0.2-0.3		Rainbow trout	1	37	Tap H_2O
	L-w	Greater than 0.3		Rainbow trout	3-4	37	Tap H_2O
	L-w	0.05-0.2		Sensitive fish	1	13	Average H_2O quality
	L-w	0.11-0.13		Fish	2	17	
	L-w	10		Roots of tomato cuttings	1	8	
	L-w	10		Cut flowers	0	8	
	L-w	50		Gerbera snap-dragon	2	8	
	L-w	50		Gladiolus and rose	0	8	
	L-w	3	1 week	Cabomba and elodea	2	8	Discolored
	L-w	5	4 days	Cabomba and elodea	4	8	
	L-w	100-500 177-354 250, 350, 1500, 1260		Plants	1-3	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Chlorine	L-w	Less than 71, less than 177, 62		Plants	0	17	
	L-w	1000	(Rapidly)	Freshwater weeds, (e.g. blanketweed)	4	26	Stream
	L-s			Peach trees	4	38	3.4 atmospheres (osmotic pressure of chlorine)
	L-w	0.5-1.0		Algae	4	17	
	L-w	5-10		Synura	4	17	
	L-w	1.0		Minute crustacea, rotifers, diatoms	4	17	
	L-w	1.0		Worms, mollusks, mites and larvae	0	17	
	L-w	0.5	72 hrs	Daphnia	4	17	Soft H_2O
	L-w	1.0		Daphnia	4	17	Nile River H_2O
	L-w	2.0		Daphnia and cyclops	4	17	
	L-w	2.6	1.5 hrs	Larvae of Chironomous	4	17	
	L-w	1.3	3.2 hrs	Larvae of Chironomous	4	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Chlorine	L-w	0.65	24 hrs	Larvae of Chironomous	4(85%)	17	
	L-w	2.5		Freshwater mussels, snails and sponges	4	17	
	L-f	0.46-4.67		Chinese holly (Ilex cornuta, Lindl) egg plant, tobacco (Nicotiana tobacum, L.)	0	43	
	L-f	0.46-4.67		Peach, coleus (Coleus blumei, Benth.), cosmos (Cosmos sulphureus, cov.) buckwheat, hybrid tea rose (Rosa sp.)	2	43	
	L-f	0.46	1 hr	Buckwheat	2	43	
	L-f	0.56	2 hrs	Coleus	2	43	
	L-f	0.56	3 hrs	Peach	2	43	
	L-f	0.56	2 hrs	Halesia	2	43	
	L-f	1.3	2 hrs	(Rhodotypos sp.)	2	43	
	L-f	1.3	1/2 hr	Bean	2	43	
	L-f	1.3	1/2 hr	Radish	2	43	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Chlorine	L-f	1.5	1/2 hr	Rose	2	43	
	L-w	50		Plants	0	8	Water and/or sprinkled
	L-w	100-150		Plants	0-2	8	Water and/or syringed
	L-w	200-300		Plants	1-3	8	Water and/or syringed
	L-w	500-1000		Seedlings	2	8	Watered and/or syringed, retarded emergence
	L-w	500-1000		Plants	3-4	8	Watered and/or syringed
	L-w	5		Plants	0	8	Grown in equal parts of sand and loam
	L-w	50, 100		Plants	1-2	8	Grown in equal parts of sand and loam
	L-w	5		Roots of tomato cuttings	0	8	
Bromine	L-w	20		Goldfish (Carassius auratus, L.)	4	26	18-23°C.
	L-w	10		Daphnia magna	4	17	Soft H_2O
	L-w	20	15-96 hrs	Goldfish (Carassius auratus, L.)	4	17	Hard H_2O

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Bromine	L-w	29		Minnows and goldfish	4	17	
	L-w	400	51 min	Fish	4	17	
	L-w	0-10	48 hrs	Fish	0	17	
Sodium Bromate	L-w	210		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O, 25°C.
	L-w	1,180 (BrO_3^-)		(Polycelis nigra)	4	17	
Sodium Bromide	L-w	4,100 8,200		(Daphnia magna)	4	17	Lake Erie H_2O, 25°C.
	L-w	11,200 (Br^-)		(Polycelis nigra)	4	17	
Nitric Acid	L-w	750	0.5-0.8 hrs	Goldfish (Carassius auratus)	0	26	pH 3.4
	L-w	200	Over 4 days	Goldfish (Carassius auratus)	0	26	pH 4.9
	L-w			Minnows (Phoxinus phoxinus)	4	26	pH 5.0
	L-w	25-36(H^+)		Fish	4	26	pH 3.3-3.15
	L-w	107		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O @ 25°C.

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Nitric Acid	L-w	0.01(H^+)		Stickleback	4	17	pH 5.0
	L-w	1.6		Trout	4	17	
	L-w	1.56-2.0		Fish	4	17	
	L-w	15.6	24 hrs	Trout	4	17	
	L-w	98.5		Pickerel	4	17	
	L-w	113		Whitefish	4	17	
	L-w	200		Minnow and goldfish	4	17	
	L-w	750	0.5-1.0 hr	Goldfish	4	17	Hard H_2O, pH 3.4
	L-w	1000	0.5 hr	Trout	4	17	Tap H_2O
	L-w	1000	7 hrs	Minnow	4	17	pH 4.4
	L-w	5.75		Minnow	0	17	pH 5.2
	L-w	5.75		Shiners	0	17	
	L-w	5.75-20		Fish	0	17	
	L-w	20		Carp, goldfish, and suckers	0	17	
	L-w	200	More than 100 hrs	Goldfish	0	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Nitric Acid	L-w	200	More than 4 days	Goldfish	0	17	pH 4.9
Sodium Nitrite	L-w	20		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O
	L-w	28(NO_2^-)		(Polycelis nigra)	4	17	
	L-w	17.1	24 hrs	Minnows	0	17	
	L-w	5000		Goldfish	0	17	Decreased toxicity of 10 ppm of $CuSO_4$
Hydrochloric Acid	L-w			Goldfish	0	26	pH 4.5
	L-w			Stickleback	0	26	pH 5.2-5.4
	L-w			Stickleback	4	26	pH 4.8, 15-18°C.
	L-w	100	4 min	Trout	2	31	Loss of equilibrium
	L-w	3.6	48 hrs	Sunfish	4	17	Distilled H_2O
	L-w	3.65	24 hrs	Carp, shiners, sunfish	4	17	
	L-w	8.0	24 hrs	Sunfish	4	17	
	L-w	10.0	24 hrs	Trout	4(50%)	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrochloric Acid	L-w	67.5		Whitefish, pickerel	4	17	
	L-w	166	4-7 hrs	Goldfish	4	17	Hard H_2O
	L-w	200		Perch, roach	2	17	Collapsed in distilled H_2O
	L-w	1000	2-5 min	Trout	2	17	Overturned in tap H_2O
	L-w	20		Minnows, goldfish	0	17	
	L-w	157	100 hrs	Goldfish	0	17	Hard H_2O
	L-w	56		(Daphnia magna)	4	17	Soft H_2O
	L-w	62		(Daphnia magna)	4	17	Lake Erie H_2O
	L-w	25-50 (acidity)		Bluegill fingerlings	4	17	pH 3.30-3.0
	L-w	4		Trout	4	17	
	L-w	166	4.5-6.5 hrs	(Carassius auratus) goldfish	0	17	pH 4.0
	L-w	159	4 days	(Carassius auratus) goldfish	0	17	pH 4.5
Hydrogen Cyanide	L-w	4-7 mg(CN)		Small potted tomato plants	3	8	Collapsed

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Hydrogen Cyanide	L-f	1100		Valencia orange trees	2	29	
	L-s	4-10mg (CN) 500gm soil		Plants	1	29	
	L-w	0.03(CN^-)		Fish	2	26	
Sodium Cyanide	L-w	0.00004N	90 min	(Gasterosteus)	2	26	32% normal oxygen consumption
	L-w	More than 0.0004N	Less than 90 min	(Gasterosteus)	4	26	
	L-w	0.00005 N	30 min	Stickleback	2	26	40% normal oxygen consumption
Cyanides (eg.KCN & NH_4CN)	L-w	0.04-0.1 (CN)		Fish	4	26	
Potassium Cyanide	L-w	0.023(CN)	5 yrs	Rainbow trout	0	26	17.5°C.
Cyanide	L-w	0.04		Trout	4	26	
Potassium Cyanide	L-w	0.4(CN)		(Carassius auratus) goldfish	4	26	21.5°C.
	L-w	0.1-0.3		(Carassius auratus) goldfish	4	26	
	L-w	0.13(CN)		(Salmo irideus) rainbow trout	4	26	5-7°C.

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Potassium Cyanide	L-w	0.07(CN)		(Salmo irideus) rainbow trout	4	26	17.5°C.
Sodium Cyanide	L-w	1.04(CN)		(Gasterosteus aculeatus) stickle-back	4	26	17°C.
	L-w	0.5-0.7	24 hrs	Minnows	4(25%)	17	
	L-w	0.75	24 hrs	Minnows	4(50%)	17	
	L-w	0.8	24 hrs	Minnows	4(100%)	17	
	L-w	1	20 min	Trout	4(100%)	17	
	L-w	2	47 min	Trout	4	17	
	L-w	3.1	90 min	Fish	2	17	Depressed respiration
	L-w	4.3		Hardy carp	3	17	Paralyzed
	L-w	5	12 min	Shiner	4	17	
	L-w	10	4 min	Shiner	4	17	
	L-w	0.02		Trout fingerlings	0	17	
	L-w	0.08		Trout	0	17	
	L-w	0.3	24 hrs	Minnows	0	17	
	L-w	2.3	155 min	Fish	0	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Sodium Cyanide	L-w	3.4		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O, 25oC.
	L-w	30		(Polycelis nigra)	4	17	
Cyanide	L-w	0.2(CN)		(Lebistes reticulates), (Melanotoenia nigraus)		37	
		0.5(CN)		(Mesogonistiers chantodan), (Tanichthys albonubis)		37	
		1.0(CN)		(Aphyocharax rubrysinnis), (Brachydanic rerio), (Phoxinus phoxinus), (Barbus cumningi), (Rasbora heteromorpha), (Salmo gairdnerii)		37	
Cyanide	L-w	0.05	120 hrs	Trout	4	26	
	L-w	0.05-0.1		Fish	4(50%)	26	
	L-w	0.1		Fish	4(50%)	26	
	L-w	0.1-0.2	1-2 days	Rainbow trout	4(50%)	26	
	L-w	0.126	170 min	Trout	2	26	Overturned
	L-w	0.15	4.8-64 min	Trout	2	26	Overturned

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Cyanide	L-w	0.176		Bluegills and sunfish	4	26	
	L-w	0.2	(Rapidly)	Fish	4	26	
	L-w	0.42	11 min	Trout	2	26	Overturned
	L-w	1.0	20 min	Trout	4	26	
	L-w	10.0	1.5 hrs	Carp	4(50%)	26	
	L-w	0.02	27 days	Trout	0	26	
	L-w	0.084		Trout	0	26	
	L-w	0.25		Bluegill	0	26	
	L-w	0.375		Bullheads	0	26	
	L-w	0.4	96 hrs	Bluegills	0	26	
	L-w	0.5	96 hrs	Bullheads	0	26	
Potassium Cyanide	L-w	0.1-0.3	3-4 days	Goldfish	4	26	Hard H_2O
	L-w	0.14	1 hr	Trout	2	26	Helpless at 5°C.
	L-w	0.25-1.0		Fish	4	26	Unaerated H_2O
	L-w	0.27	10-120 min	Trout	2	26	Overturned
	L-w	0.78	43-118 hrs	Goldfish	4	26	Distilled H_2O

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Potassium Cyanide	L-w	15.0		Tadpoles	4	26	
	L-w	1.0		Freshwater mussels	2	17	Closed and lost ability to attach to fish
	L-w	40		Animal plankton	4(some)	17	
	L-w	200		Animal plankton	4(all)	17	
	L-w	65		(Daphnia magna)	4	17	
Sodium Cyanide	L-w	0.05	124 hrs	Trout	4	17	
	L-w	0.4	1 hr	Minnows	1	17	Stopped eating
Ammonia	L-f	40	1 hr	Tomato, sunflower and coleus	1	43	
	L-f	16.6	4 hrs	Tomato, sunflower and coleus	1	43	
	L-f	8.3	5 hrs	Tomato, sunflower and coleus	0	43	
	L-w	1.2-3	Rapidly	Relatively hard fish	4	13	
	L-w	2-7		Some fish	4	13	Certain types of water
	L-w	2.9	13 hrs	(Cichla ocellaris)	4	28	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Ammonia	L-w	5.6	13 hrs	(Astonotus ocellatus)	4	28	
	L-w	9.6	13 hrs	(Piaba) (Characinid)	4	28	
	L-w	5.2	13 days	(Piaba)	4(47.8%)	28	
	L-w	50	47 min	Trout	4	31	
	L-w	13(NH_4OH)		Fish	4	31	
	L-w	2-2.5	1-4 days	Goldfish (Carassius auratus)	4	26	
	L-w	15		Perch (Perca fluviatilis)	4	26	
	L-w	2-25		Various spp. (fish)	4	26	
	L-w	0.3-1.0		Fish	2-4	17	
	L-w	2.0		Fish	2-4	17	
	L-w	2.5		Fish	2-4	17	pH 7.4-8.5
	L-w	6.3		Trout	2-4	17	
	L-w	7-8	1 hr	Sunfish	2-4	17	
	L-w	13		Fish	2-4	17	
	L-w	17.1	1 hr	Minnows	2-4	17	

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Compound	Type of Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Ammonia	L-w	1.5		Most varieties of fish	0	17	
	L-w	4.3	1 hr	Minnows	0	17	
	L-w	0.4-0.5		(Aphanizomenon)	4	17	
Ammonium Hydroxide	L-w	6.25	24 hrs	Brook trout	4	17	
	L-w	10		Carp, trout, suckers, shiners	4	17	
	L-w	13	24 hrs	Suckers, shiners and carp	4	17	
	L-w	20	15 min	Suckers, shiners and carp	4	17	
	L-w	30	24 hrs	Small fish	4	17	
	L-w	9.4	24 hrs	Suckers, shiners and carp	0	17	
	L-w	Less than 8.75		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O, 25°C.
Ammonia	L-w	1.56(N)	13 days	Fish	4(30%)	37	H_2O saturated with O_2
	L-w	1.33(N)	40 min	Fish	4(100%)	37	H_2O, 46.9% saturated with O_2
Aluminum	L-w	0.07		Stickleback	4	17	

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Aluminum	L-w	0.5		Fish	4	17	Alkaline H_2O
	L-w	5.0	5 min	Trout	4	17	
	L-w	0.25		Fish	0	17	Acid H_2O
	L-w	1.0	5 min	Trout	0	17	
	L-w	190.0		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O
Aluminum Ammonium Sulfate	L-w	523	10 hrs	Trout	4	17	Tap H_2O
Aluminum Chloride	L-w	Less than 6.7	64 hrs	(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O, 25°C.
	L-w	Greater than 0.5 (Al)		Goldfish and others	4	13	pH 7.2-7.4, Tap H_2O
	L-w	2.7 (Al)	3.6 hrs	Young eels	4	13	
	L-w	0.27 (Al)	50 hrs		0	13	
	L-w	44	11-14 days	Fish	0	13	Sea H_2O
	L-w	88	30 min-9 days	Fish (marine)	4	13	Sea H_2O
	L-w	132-176	4 days	Fish	4	13	Sea H_2O

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Aluminum Nitrate	L-w	110 (Al)		(Polycelis nigra)	4	13	
	L-w	0.55		Stickleback	4	26	Tap H_2O
	L-w	0.1 (Al)		Stickleback	4	26	15-18°C.
	L-w	0.1 (Al)		Fish	4	13	Soft H_2O, pH 6.0-6.2
Aluminum Potassium Sulfate	L-w	206		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O
	L-w	10	4 days	Goldfish	4	17	Hard H_2O
	L-w	100	12-96 hrs	Goldfish	4	17	Hard H_2O, pH 6.8
	L-w	10	96 hrs	Goldfish	0	17	pH 7.6
	L-w	100		Goldfish	4	26	18-23°C.
	L-w	250		Minnows	4	17	
	L-w	544	6 hrs	Salmon	4	17	Tap H_2O
	L-w	544	15 hrs	Trout	4	17	Tap H_2O
	L-w	1000	1-10 hrs	Goldfish	4	13	Hard H_2O, pH 5.5
	L-w	10	100 hrs	Goldfish	0	13	Hard H_2O
	L-w	0.1 (Al)		Fish	4	13	Soft H_2O
Aluminum Sulfate	L-w	17.1	1 hr	Minnows	0	17	Tap H_2O

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Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Aluminum Sulfate	L-w	100	7 days	Goldfish, sunfish, and bass	0	17	
	L-w	250	8-24 hrs	Goldfish, sunfish, and bass	4	17	
	L-w	0.1		Fish	4	13	Soft H_2O, pH 6.0-6.2
	L-w	1 (Al)	48 hrs	Trout	0	13	Tap H_2O
	L-w	5 (Al)	5 min	Trout	2	13	Overturned, pH 6.8
	L-w	7(SO_4)	5 days	Mummichogs	4	13	Fresh H_2O
	L-w	100	7 days	Bluegills, sunfish, blackbass, goldfish	0	13	Moderate hard H_2O, pH 5.6
	L-w	250	8-24 hrs	Bass, goldfish	4	13	Moderate hard H_2O, pH 4.5
Boron	L-s	Less than 4		Sensitive plants	0	17	
	L-s	20-100		Alfalfa, date palm	0	17	
	L-w	0.5-1.0		Plants	2	4	Toxic
Boric Acid	L-w	2000		Rudd and rainbow trout	0	17	
	L-w	5000		Rainbow trout	1	17	

(continued)

(continued)

Compound	Type of* Study	Concen. ppm**	Time of Exposure	Organism(s)	Injury ***	Ref.	Remarks
Boric Acid	L-w	80,000	Few min	Rainbow trout	2	17	Immobilized and lost balance
	L-w	80,000	Few min	Rudd	0	17	
	L-w	6250	18 hrs	Rudd	4	17	
	L-w	Small amount		(Proteus vulgaris)	2	33	Prevented swarming
Lithium	L-w	0.045-0.08		Citrus trees	2	34	Toxic
	L-w	Less than 50		Fish	0	13	
	L-w	617 (Li)	24.5 hrs	Fish	4	34	
	L-w	320-400	Rapidly	Goldfish	4	34	Moderate temperature
Lithium Chloride	L-w	320-620 (Li)	1 day	Freshwater fish	4	13	
	L-w	Greater than 7.2		(Daphnia magna)	2	17	Immobilized, Lake Erie H_2O
	L-w	3-750	22-27 hrs	Goldfish	4	17	Distilled H_2O

TABLE A-2. Comparative susceptibility of various species to HF.*

Time of Exposure	Ave. Conc. ppm	Buckwheat	Sweet Potato	Peach	Tomato	Corn	Bean	Pine
2 hours, 10 min	1.48	3a	3a	3a	3a	3a	3a	3a
2 hours, 10 min	0.67	3	3	3	1	3	1	3a
4 hours	0.27	3	2	2	0	3	0	3a
7 hours, 30 min	0.135	3	3	3	0	3	0	3a
4 hours	0.13	1	1	1	0	1	0	2
6 hours, 40 min	0.085	1	1	1	0	0	0	2
4 hours	0.07	0	0	0	0	0	0	1

* Table from Zimmerman, Effects on Plants of Impurities Associated with Air Pollution. (43)

Legend: Extent of Injury
0 - no injury
1 - slight
2 - moderate
3 - considerable
3a - severe
4 - lethal

TABLE A-3. Effect of light and dark on HF toxicity.*

Plant	Daylight Exposure						Darkness Exposure			
	1.5 ppm HF		5 ppm HF		10 ppm HF		1.5 ppm HF		5 ppm HF	
	Hours	Injury	Hours	Injury	Hours	Injury	Hours	Injury	Hours	Injury
Corn (Zea mays)			149.4	X						
Locust (Robinia pseudoacacia)	141.3	...	101	...	50.1	...				
Squash (Cucurbita maxima)			126.2	X	42.1	X				
Tomato (Lycopersicon esculentum)			65.4	X	30.2	X				
Apple (delicious)(Malus sylvestris)	237.3	X	33.0	X	25.8	X	224.0	X	97.2	X
Pine (Pinus ponderosa)	180.7 367.3	... X	42.6	X	18.7	X	241.3	...	73.6	X
Willow (Salix aurea)	151.3	X	87.6	X	34.0	X	54.7	X	73.6	X

These results are based on constant flow fumigation using leaves that had matured for 2 or 3 months prior to experimentation.

Legend: X - plant removed from fumigation upon initial observance of foliar burn.

... - no visible burn at completion of experiment.

* Table from Adams, Relationship Among Exposure Periods, Foliar Burn and Fluorine Content of Plants Exposed to Hydrogen Fluoride. (1)

TABLE A-4. Effect of chlorine on bacteria.*

Dose of chlorine added (ppm)	5.0	5.5	6.5	7.0	7.5	10.0
Number of tests	7	3	3	6	5	6
% bacteria destroyed**	30-99.4	68-99.5	98-99.7	89-99.8	90-99.8	99-99.7

* Mason, Water Supply. (31)
** In about 3 hours.

TABLE A-5. Effect of dosing clear water artificially seeded with ordinary bacteria with 0.5 ppm of available chlorine.*

Time between dosing and sowing (min.)	0	5	30	60	120
Number of bacteria/cc	220,000	1,200	800	15	2

* Mason, Water Supply. (31)

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The effects of 21 missile fuel components on aquatic organisms, soil microflora, plants and soils were determined. Goldfish and Daphnia were subjected to 0, 1, 10, 100 and 1000 ppm of the test compounds for 72 hours in the aquatic studies. Some or all of 10 goldfish and 13 Daphnia died, when exposed to 100 ppm of the test chemicals. Counts of bacteria, actinomycetes, and fungi in the soil microflora

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I. AFSC Project 6302, Task 630204
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studies showed no significant decrease in any of the organisms with a 100 ppm application of test chemical to the soil samples. Three of the chemicals may sterilize the soil of actinomycetes. Plant studies were threefold using squash, soybean, cotton, cowpea and corn; germination studies using 1000 ppm of each test chemical produced consistent inhibition of germination by two of the compounds and two ionic species; seedling studies using a soil drench of each test chemical at 100 ppm, produced toxic symptoms with three of the ionic components; seedling studies using three test chemicals (gases) at 100 ppm as air pollutants produced severe injury to death of all species with each of the test gases. Soil studies (1000 ppm) included the leachability and runoff potential of each test chemical as well as the effects on soil.

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